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EVALUATION AND DEVELOPMENT OF A CAPNODYNAMIC METHOD FOR ESTIMATION OF PULMONARY BLOOD FLOW IN A PORCINE MODEL

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Evaluation and development of a capnodynamic method for estimation of pulmonary blood flow in a porcine model

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Stockholm 2015

In loving memory of my grandmother
Siri Karolina Lindevall
-strong and bright with a heart of gold

"No monitoring device, no matter how accurate or complete, would be expected to improve patient outcome, unless coupled to a treatment that itself improves outcome"

Michael R Pinsky

ABSTRACT

Effective pulmonary blood flow (EPBF) i.e. cardiac output (CO) minus shunt flow could be estimated by a capnodynamic equation. Via introduction of short pauses in the ventilatory pattern the required alterations of carbon dioxide are induced. By integrating the alterations into the equation EPBF could be calculated. The mathematical formula also includes an equation term, effective lung volume (ELV) that has previously been shown to correlate to FRC.

The capnodynamic method (CO_{EPBF}) was evaluated during haemodynamic and ventilatory alterations in a porcine model before and after lung lavage. An ultrasonic flow probe positioned around the pulmonary trunc (CO_{TS}) was used as a reference method for CO. ELV was compared to the sulphur hexafluoride (SF6) method and stability during CO alterations was assessed. Two different ventilation patterns were studied, in papers I-III a pattern based on inspiratory holds and in paper IV a pattern based on expiratory holds. Bland Altman statistics were used for evaluation of the agreement for absolute values and the four-quadrant and polar plot methodologies were used to assess trending ability.

CO_{EPBF} based on inspiratory holds showed good agreement and trending abilities at PEEP 5 cmH_2O. However, a paradoxical increase was seen when PEEP was increased to 12 cmH_2O.

Lung lavage resulted in a significant decrease in lung function with a two-fold increase in shunt fraction and the performance of CO_{EPBF} was significantly impaired. However, the trending ability was largely preserved when assessed by the four-quadrant methodology.

The shunt fraction was dependent of CO regardless of the degree of lung injury and PEEP level. When shunt levels were > 20%, CO_{EPBF} underestimated CO_{TS}.

ELV was not significantly affected by CO alterations at an unchanged PEEP level. A small difference between ELV and FRC_{PEEP} was seen at PEEP 5 cmH_2O before lavage and at PEEP 12 cmH_2O after lavage.

Since a ventilatory pattern based on inspiratory holds is likely to affect the pulmonary capillary blood flow *per se* a modification of the pattern might improve the performance. A ventilatory pattern based on expiratory holds could be assumed to reduce this variation. In paper IV, when CO_{EPBF} was based on expiratory holds, this paradoxical increase in CO_{EPBF} was not displayed and the performance was improved.

This capnodynamic method could be considered a non-invasive alternative for estimation of EPBF and ELV in mechanically ventilated subjects without significant lung pathology. Considering that the ability to detect trends is good, this device might have the prerequisite to be used to guide goal-directed treatment protocols for CO optimisation.

Keywords; cardiac output, pulmonary blood flow, lung lavage, shunt flow, carbon dioxide, alveolar, hemodynamic, porcine model, sulphur hexafluoride, pulmonary trunc, mechanical ventilation, goal directed therapy

LIST OF SCIENTIFIC PAPERS

This thesis is based on the following papers, which will be referred to in the text by their roman letters.

I. **Novel continuous capnodynamic method for cardiac output assessment during mechanical ventilation**

Hällsjö Sander C, Hallbäck M, Wallin M, Emtell P, Oldner A, Björne H

British Journal of Anaesthesia 2014 May; 112(5):824-31

II. **A novel continuous capnodynamic method for cardiac output assessment in a porcine model of lung lavage**

Hällsjö Sander C, Hallbäck M, Suarez Sipmann F, Wallin M, Oldner, Björne H

Acta Anaesthesiologica Scandinavica 2015 Sep; 59(8):1022-31

III. **Capnodynamic assessment of effective lung volume during cardiac output manipulations in a porcine model**

Hällsjö Sander C, Lönnqvist P-A, Hallbäck M, Suarez Sipmann F, Wallin M, Oldner A, Björne H

Submitted

IV. **An improved capnodynamic method for continuous assessment of effective pulmonary blood flow**

Hällsjö Sander C, Sigmundsson T, Hallbäck M, Suarez Sipmann F, Wallin M, Oldner A, Björne H

Manuscript

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LIST OF ABBREVIATIONS

BL	Baseline
C_aCO_2	Systemic arterial carbon dioxide concentration
C_CCO_2	Pulmonary end-capillary concentration of carbon dioxide
C_VCO_2	Mixed venous concentration of carbon dioxide
CE	Coefficient of error
CO	Cardiac output
CO_{EPBF}	Effective pulmonary blood flow as assessed by the capnodynamic method
CO_{PAC}	Cardiac output as assessed by the pulmonary artery catheter
CO_{TS}	Cardiac output as assessed by the ultrasonic flow probe
CO_{TPT}	Cardiac output as assessed by the transpulmonary thermodilution method
CV	Coefficient of variation
Δt^n	The duration of each breathing cycle
ELV	Effective lung volume
EPBF	Effective pulmonary blood flow
F_ACO_2	Alveolar fraction of carbon dioxide
F_iO_2	Inspired fraction of oxygen
Fr	French
FRC_{PEEP}	Functional residual capacity at a positive PEEP
GDT	Goal directed therapy
L_{gas}/L_{blood}	Litre gas per litre blood
L/min	Litre per minute
LoA	Limits of agreement
MAP	Mean arterial pressure
n	Current breath
n^{-1}	Previous breath
NICE	National Institute for Health and Care Exchange
PAC	Pulmonary artery catheter
PE	Percentage error
PEEP	Positive end-expiratory pressure
P/F ratio	Partial pressure oxygen in arterial blood divided by inspired fraction of oxygen
p_aO_2	Systemic arterial partial pressure of oxygen
p_aCO_2	Systemic arterial partial pressure of carbon dioxide

p_ACO_{2ET}	Alveolar end-tidal partial pressure of carbon dioxide
p_ACO_2	Alveolar partial pressure of carbon dioxide
SCO_2	Coefficient of solubility for carbon dioxide in blood
SD	Standard deviation
SEM	Standard error of the mean
SF6	Sulphur hexafluoride
SV	Stroke volume
SVR	Systemic vascular resistance
VCO_2	Quantity of carbon dioxide eliminated from the lungs
$VTCO_2^n$	Quantity of carbon dioxide exhaled by the n^{nt} tidal volume
VTI	Velocity time integral

INTRODUCTION

PROLOGUE

Once upon a time in a fairly large town a young and enthusiastic doctor started working in a hospital. From dusk till dawn and sometimes even during the night she tended to patients in the operating theatres and pondered the variations of the numbers displayed on the patient monitors. She asked the older doctors about those numbers and what they really meant. Although they discussed these variations and possible treatments she did not really understand as much as she wished. She went home to her house and read the old book she used to study in school. Despite her best efforts it was not possible to find an answer and the older doctors could not help her anymore.

The young doctor often reflected upon something frightening. Some of the patients she tried her best to look after in the operating theatre became ill and sometimes they died before leaving the hospital. She wondered, "Could I do something better to prevent this?" Sometimes the other doctors and nurses told her that she pondered too much for her own good.

The still young but now a bit more experienced doctor was curious and started to search for guidance from other sources. She was now really frustrated because she realised that she had to learn more to be able to even understand what the numbers really meant. To acquire that knowledge the fairly young doctor moved to the large hospital in town.

The doctors and nurses at the large hospital welcomed her. They seemed to like her pondering although the numbers on the patient monitors varied as much here as at her last hospital. The now a bit more experienced doctor realised that all questions don't have answers, but with the right skills, hard work and funding from the king himself she might be able to contribute some answers on her own.

The not really young doctor began her journey as a researcher gaining the skills to understand other sources, doing experiments, writing science, and counting statistics: in the end hoping to contribute with new knowledge into the world. You could say that research resembles the fairy tale of frogs, kisses and finding the prince. A lot of hypotheses have to be formulated and tested before you have the result you desire.

Finally, the former young doctor who was convinced that she preferred to work as a clinician realised that research is a prerequisite for improving care for patients.

So, this is not the end of the story, this is just the beginning of a new fairy tale.

Enjoy!

CARDIAC OUTPUT AND CIRCULATION

1628 William Harvey described his discovery of the circulation of blood (1) "*Exercitatio Anatomica de Motu Cordis et Sanguinis in Animalibus*" which translates to "An Anatomical Exercise on the Motion of the Heart and Blood in Living Beings". Since then the function of the heart and circulatory system have been further assessed and described in detail. Several methods for cardiac output (CO) monitoring have been developed and are used in haemodynamically unstable patients in intensive care units and operating theatres.

This subject is of great interest for perioperative and intensive care physicians resulting in numerous studies describing the advantages and disadvantages of new and established CO monitors (2, 3). Cardiac output monitoring has also been assessed in combination with predefined goal directed protocols. Several studies display a decreased morbidity rate and sometimes even decreased mortality when CO monitoring was used to guide CO optimisation (4-6).

CARDIAC OUTPUT MONITORING IN THE PERIOPERATIVE SETTING

Approximately 230 million patients undergo surgery world wide every year (7). In Europe and the United States > 10% of patients undergoing surgical procedures run an increased risk of major complications. Approximately up to 5% die before being discharged from the hospital (8-11). This group of patients is defined as a high-risk population for perioperative complications and accounts for 80% of postoperative deaths (12). Preventing postoperative complications is crucial in order to reduce morbidity and mortality and is also cost-effective (13).

High-risk surgical patients seem to have less capacity to adjust to the increased demand of oxygen delivery associated with surgical trauma in the perioperative period (12, 14). Unfortunately, standard peroperative monitoring is limited to basic haemodynamic parameters including heart rate, blood pressure and saturation, which have been shown to correlate poorly to flow-based vital parameters and oxygen delivery (15). In contrast CO is closely associated with oxygen delivery and tissue perfusion (16, 17).

In perioperative high-risk surgical patients CO monitoring is essential to guide fluid, vasopressor and inotropic therapy to optimise oxygen delivery. In this population CO monitoring and subsequent optimisation with goal directed therapy (GDT) protocols has been shown to reduce the risk of postoperative complications. Despite this, CO monitoring is not widely used in the perioperative setting (18-21).

Intubation and mechanical ventilation is commonly required during general anaesthesia. Suboptimal ventilator settings might negatively affect both ventilation and perfusion of the lung and could be potentially harmful (22). Standard monitoring in ventilated patients assesses pulmonary pressures and volumes without taking the pulmonary circulation into account. A method integrating monitoring of both pressures and perfusion in the lung could be an attractive tool for optimisation of ventilator settings.

CARDIAC OUTPUT MONITORING

Thermodilution

The pulmonary artery catheter (PAC) (Edwards Lifesciences, Irvine, CA, USA) is considered to be the clinical reference method for CO monitoring. The device can be calibrated manually by injection of a known volume with a known temperature into the right atrium via the proximal injection port. The injected volume is carried by the circulation and diluted (23). A thermistor placed downstream in the circulation senses the change in temperature and CO can be calculated. The inherent precision of the method is improved if three bolus injections are performed and an average of these are used for calculation of CO (24). Commonly an automated mode induces intermittent heating of an electric filament attached to the PAC approximately 15 to 25 cm proximal from its tip for continuous CO monitoring. The subsequent series of heat signals from the thermistor on the tip are used to generate a thermodilution curve (23).

Limitations include the complications described in the literature associated with invasiveness (25). The system requires a specially designed catheter inserted via a central vein guided by pressure signals through the right ventricle into the pulmonary artery. Insertion of the device and interpretation of the data requires a trained operator (23, 25).

Besides being considered the clinical reference method for CO monitoring advantages include the opportunity for additive information, such as estimation of pulmonary vascular pressures and mixed venous gas saturation (23). Patient outcome data when the PAC was used to guide CO optimisation are not uniform and the use of the device is declining. However, some authors argue for an increased use in selected patients groups, for example patients with a severely impaired cardiac function due to an acute illness (25-27).

Ultrasound and Doppler techniques

The oesophageal Doppler measures blood flow velocity in the descending thoracic aorta by means of a Doppler transducer placed at the tip of a single-use probe. The probe is inserted via the oesophagus until a characteristic aortic velocity signal is found. According to the pulse wave Doppler principle the distance the blood travels during systole equals the velocity time integral (VTI). Stroke volume (SV) and CO are calculated from an average of usually five VTI values and the cross sectional area of the aorta. The CardioQ device (Deltex Medical, Chichester, West Sussex, UK) uses a validated population-based internal chart for estimation of the cross sectional area of the aorta in correlation to the sex, weight, height and age of the patient (28).

Limitations are the necessity for manual manipulation of the probe position since a suboptimal angle of insonation decreases the accuracy of the method. A poor signal is often associated with an underestimation of CO. A reoccurring problem in the operating room is interference with the Doppler signal from the electric cautery. The position of the probe only permits measurements of blood flow to the lower parts of the body. This has been accounted for in the algorithm assuming the distribution to be 70% of CO to the lower parts of the body. A potential source of error is if this relation is altered (28-30). Pathology in the oesophagus is a contraindication for inserting a probe.

Several studies have shown a positive impact on outcome with a reduced length of stay in the high-risk surgical population (29-34) and the method is recommended by the National Institute for Health and Care Exchange (NICE) for use in patients undergoing high-risk surgery or other surgical patients in whom the clinician would consider using invasive CO monitoring. However, the positive effect on outcome has been questioned in more recent studies (35).

The Ultrasonic Cardiac Output Monitor (USCOM, Sydney, Australia) is a non-invasive ultrasound probe measuring CO at the sternal notch (trans aortic blood flow) or at the left parasternal position (transpulmonary blood flow). The area of the outflow tract is estimated by a nomogram based on the subject's height. Stroke volume is calculated as a product of VTI and the estimated cross sectional area (2, 28).

Potential limitations and sources of error are associated with estimation of the outflow tract area by a chart or obtaining a suboptimal flow signal. Both the operator and the subject could trigger this suboptimal result. An advantage of this method its total non-invasiveness. The accuracy of the method when compared to thermodilution varies between high and poor (36, 37).

Echocardiography

Measurements can be obtained by a transoesophageal or a transthoracic view and SV can be measured in two different ways. The volumetric approach by estimating the end-systolic and end-diastolic left ventricular volume and subtracting one from the other or by applying the Doppler technique multiplying VTI with the cross sectional area of the left ventricular outflow tract (2, 3). This technique has been described as an ideal CO monitor because of precise estimation of CO in combination with the ability to estimate other aspects of cardiac performance like ventricular and valvular function (3). However, like the other ultrasound/Doppler methods it is unsuitable for continuous CO monitoring. The high costs associated with the equipment and the considerable need for education limits the use of echocardiography (3).

Thoracic bioimpedance and bioreactance

Cardiac output is calculated through analysis of variation in voltage in response to a high frequency transthoracic current (3). Several studies comparing bioimpedance with thermodilution show poor agreement (38-40). In a later study with updated versions of the method the results improved (41). However, there seems to be several limitations with this technique. Decreased accuracy is associated with volume overload and increased amount of extravascular lung-water, variations of patient size, temperature, humidity and the positioning of the electrodes (2).

Bioreactance technology processes the impedance signal in a different way by measuring the phase shift in voltage across the thorax. When compared to thermodilution and pulse contour analysis the results are promising (42-44). Both methods are entirely non-invasive.

Pulse contour analysis

Arterial waveform analysis is based on the relation between blood pressure, SV, arterial compliance, and systemic vascular resistance (SVR) (45). The major limitations are haemodynamic instability, cardiac arrhythmias and other factors disturbing the arterial waveform such as changes in the vascular resistance (45).

Calibrated pulse contour analysis

Two methods based on pulse contour analysis in combination with transpulmonary thermodilution with saline for calibration are available; PiCCO (Pulsion, Munich, Germany) and the more recently introduced Volume view/EV1000 (Edwards Lifesciences, Irvine, CA, USA). Transpulmonary thermodilution have been shown to be a reliable alternative to the PAC, however, the methodology is invasive and requires a central arterial and venous line (23).

The PiCCO methodology is based on the principle that SV is proportional to arterial pulse pressure and inversely proportional to arterial compliance. Cardiac output is calculated by dividing the area under the systolic part of the arterial curve by the aortic compliance. During calibration with transpulmonary thermodilution the aortic compliance and resistance are estimated and updated. Between calibrations those values are reassessed by an algorithm based on the arterial pressure curve (46). The transpulmonary thermodilution technology provides the clinician with additional values, for example extra-vascular lung water and global end-diastolic volume (47)

Clinical limitations includes a need for frequent calibrations especially when the arterial resistance changes and the requisite for a central arterial and venous line (46).

A pulse contour analysis combined with lithium chloride transpulmonary dilution for calibration (LiDCOgroup, Cambridge, UK) is less invasive than devices calibrated with saline considering the technology only requires a peripheral arterial and venous line.

The performance of this pulse contour analysis is comparable with other pulse contour techniques (2, 48) and the results are equivalent when the lithium indicator dilution technique is compared to thermodilution (49).

The limitations include requirement for recalibration after haemodynamic changes (2). Furthermore, muscle relaxants (vecuronium and pancuronium) interact with lithium chloride and decreases the accuracy of calibration after muscle relaxant has been administered. Patients treated with lithium or pregnant women in the first trimester should not be monitored with LiDCOplus. Additionally, injection of lithium chloride in not authorised in all countries (46, 50).

Non-calibrated monitors

The basic principle of FloTrac/Vigileo (Edwards Lifesciences, Irvine, CA) is the linear relationship between pulse pressure and SV. Arterial compliance and resistance is estimated from a validated nomogram and morphological patient data; sex, age, height and weight that has to be programmed into the system (45, 46). Data points are collected from the arterial

waveform and pulse pressure is calculated from the standard deviation of those (45). The system is operator independent and requires only a peripheral arterial line connected to the special pressure transducer. The most recent software showed improved performance especially in normal- and hypodynamic states with a decreased percentage error (PE) compared to earlier versions. However, in hyperdynamic states and during infusion of vasopressors the system performed poorly (51, 52).

The ProAQT/Pulsioflex (Pulsion, Munich, Germany) is a recently introduced device similar to the FloTrac but the software for pressure waveform analysis is different and a new algorithm providing an auto-calibration calculates the initial CO value. It is possible to reset this value by calibration manoeuvres and add a value obtained by another CO monitor (46, 52). This device was recently introduced so the numbers of studies are limited. However, the main findings display ability to track changes comparable to FloTrac but a poor agreement for absolute values. The auto-calibration did not improve the performance. (52).

LiDCOrapid (LiDCOgroup Cambridge, UK) uses the same algorithm as the LiDCOplus but a nomogram assesses the aortic compliance abolishing the need for calibration. The method has similar limitations as the other non-calibrated CO methods with decreased reliability when the vascular tone changes rapidly (53).

The pressure recording analytical method (PRAM) (Mostcare; Vytech Health, Padova, Italy) calculates CO based on the area under the arterial pressure curve, the analytical description of the arterial pressure waveform and the instantaneous acceleration of the arterial vessel cross-sectional area. This device does not require patient data or calibration and is semicontinuous (53). The reliability for absolute values is low (53).

The Nexfin (Edwards Lifesciences, Irvine, CA, USA) provides continuous values obtained by an inflatable finger cuff. Stroke volume is calculated by dividing the pulsatile systolic area of each beat by the computed aortic compliance. Gender, age, height and weight are programmed into the system and the algorithm is developed on the basis of a large human database including non-invasive and invasive arterial pressure tracings together with thermodilution. The performance was found to be accurate when used in intraoperative patients but declines in septic haemodynamic unstable patients in the intensive care unit (3).

CALCULATION OF CARDIAC OUTPUT BY FICK'S EQUATION

Fick's equation

Adolf Fick published an article in 1870 describing how CO could be calculated by integrating the oxygen or carbon dioxide content from a sample of pulmonary arterial and systemic arterial blood into an equation (54) (Figure 1).

Fick's principle was validated in humans in 1930 when researchers obtained samples of a mixed venous gas from brave volunteers by puncturing the right ventricle with a spinal tap needle via the thoracic wall (55) (Figure 2).

The introduction of heart catheterisation in 1940 permitted access to the pulmonary artery and Fick's principle became a clinically applicable method in humans (56). Estimation of pulmonary blood flow from the uptake of oxygen or elimination of carbon dioxide is the oldest known methodology for calculation of CO.

Figure 1. Painting of Adolf Fick (1829-1901).

$$CO = \frac{VCO_2}{CvCO_2 - CaCO_2}$$

Figure 2. Fick's classical equation states that the amount of carbon dioxide eliminated by the lungs equals the amount being transferred to the lungs by the blood during steady state conditions. CO expresses the pulmonary blood flow, VCO_2 equals the elimination of carbon dioxide by the lungs, C_VCO_2 the mixed venous content of carbon dioxide and C_aCO_2 the systemic arterial carbon dioxide content.

Indirect Fick's method

In contrast to the invasive sampling of mixed venous and arterial blood gases, analyses of expired gases is non-invasive and can be accomplished continuously. Analysis of expired carbon dioxide could be used for calculation of pulmonary blood flow by an indirect Fick's principle. This approach is dependent on induced alterations of the alveolar concentration of

carbon dioxide and allows semicontinuous calculations of effective pulmonary blood flow (EPBF) i.e. CO minus intrapulmonary shunt (57-59).

Three main principles have been described for induction of the required alterations of carbon dioxide; 1) change of alveolar ventilation 2) alter dead space and 3) add carbon dioxide to the inspiratory gas (57, 59).

When the carbon dioxide concentration is altered in a predefined way the equation could be further developed and rewritten describing two different levels of ventilation, before (b) and after (a) a change of ventilation was induced.

Equation 1 $$\boldsymbol{VCO_2[b] = EPBF \cdot (CvCO_2 - CcCO_2[b])}$$

Equation 2 $$\boldsymbol{VCO_2[a] = EPBF \cdot (CvCO_2 - CcCO_2[a])}$$

Equation 1 describes the mathematical association between the measured elimination of carbon dioxide (VCO_2) [L/min] and EPBF [L/min] multiplied with the difference between the mixed venous concentration of carbon dioxide (C_vCO_2) [L_{gas}/L_{blood}] and concentration of carbon dioxide in the pulmonary end-capillary arteries (C_cCO_2) [L_{gas}/L_{blood}]. Equation 2 describes the same relation after an alteration of ventilation has been induced.

If the mixed venous carbon dioxide concentration is assumed to be constant when measurements are obtained the equation could be further developed.

$$\boldsymbol{EPBF = \frac{(VCO_2[b] - VCO_2[a])}{SCO_2 \cdot (p_ACO_2[a] - p_ACO_2[b])}}$$

This allows calculation of EPBF from quantities measureable in the expired gas. The coefficient of solubility for carbon dioxide in blood SCO_2 [L_{gas}/L_{blood}/mmHg] is integrated into the equation to convert the alveolar partial pressure [mmHg] to concentration of carbon dioxide [L_{gas}/L_{blood}]. The partial pressure of carbon dioxide in the pulmonary end-capillary arteries is estimated from the measured alveolar concentration of carbon dioxide.

Via the development of modern ventilators with software control this old Fick's principle, when modified, could offer an alternative for estimation of EPBF. This approach could be considered non-invasive in the large group of patients requiring intubation and mechanical ventilation during general anaesthesia (7, 60, 61).

Changing alveolar ventilation

In 1980 Gedeon *et al.* described an indirect Fick method for bedside determination of EPBF in intubated and mechanically ventilated subjects. The method was evaluated in anaesthetised dogs during baseline (BL) conditions and subsequent controlled bleeding and in patients

anaesthetised for cardiac surgery. The results in dogs when compared to thermodilution were encouraging. However, the results in patients were not stated in the article (62).

The basic idea described was to induce intermittent cyclic changes in the alveolar concentration of carbon dioxide while the mixed venous concentration of carbon dioxide remained constant. A variable pause, a short breath hold between inspiration and expiration, generated periods of hypo- and hyperventilation leading to changes in minute ventilation and subsequent rapid changes in alveolar end-tidal partial pressures of carbon dioxide (p_ACO_{2ET}) and VCO_2. With this approach the tidal volume, inspiratory and expiratory time remained constant, a precondition for minimal perturbation of the pulmonary blood flow *per se*. The method required 15 minutes of recovery between measurements to secure steady state conditions. Keeping the expiratory time constant allowed p_ACO_{2ET} to be recorded at comparable points of the alveolar plateau (62).

This was followed by a publication in 2002 when a modified mathematical formula and breathing pattern was evaluated. In the previous settings the required perturbation of gas exchange was maintained for typically 15-30 seconds. An alternative approach based on a cyclic reoccurring single short breath hold was likely to induce fewer disturbances to the physiology. Including 1-3 subsequent normal breaths, until p_ACO_{2ET} is re-established, could be an attractive alternative to longer lasting perturbations. The benefit from including the information from the recovery phase was studied in patients anaesthetised for cardiac surgery (60).

In order to separate the gas exchange between the pulmonary blood and the alveoli from effects due to changes in the carbon dioxide stores in the lung tissue a formula for calculating the effective lung volume (ELV) was introduced into the equation. In this study it was suggested that ELV corresponds to the volume in the alveoli where carbon dioxide is distributed.

Adding dead space

In 1988 Capek *et al*. described a semicontinuous approach for calculation of CO by variation of dead space. This method was evaluated during alterations of CO and after induction of pulmonary oedema in dogs. A key finding was that p_ACO_{2ET} increased exponentially although the concentration of carbon dioxide in mixed venous blood remained stable during partial rebreathing. The agreement was acceptable when compared to thermodilution.

Normal ventilation was followed by 30 seconds of partial rebreathing providing EPBF values every 3.5 minutes and p_ACO_{2ET} was used as a substitute for pulmonary end-capillary concentration of carbon dioxide although corrected for dead space. In this mathematical approach the effect of the haemoglobin level of the dissociation curve for carbon dioxide was added into the equation (63).

In 1995 Gedeon's mathematical formula was evaluated by intermittent increase of dead space. This approach was evaluated both in intubated patients and after extubation. The post mechanical ventilation measurements were obtained through a mouthpiece. In contrast to the high performance during mechanical ventilation the measurements during spontaneously breathing showed less reliability due to technical problems (64).

Adding dead space in the clinical setting

The non-invasive cardiac output monitor (NICO Respironics, Murrysville, PA) is designed for use in mechanically ventilated patients. It requires integration of a specially designed circuit for automated partial rebreathing between the endotracheal tube and the Y-piece. Varying serial dead space through cyclic reoccurring opening and closing of the valve to the extra circuit induces the required alterations of p_ACO_{2ET} and VCO_2. A recording cycle lasts for approximately three minutes inducing semicontinuous measurements. The period of rebreathing lasts for 50 seconds in the former version of software but has been shortened to 30 seconds in the latest version (5.0) (65). Most studies have been performed with the former version (Figure 3).

Figure 3. The NICO loop and valve for rebreathing connected between the Y-piece and the endotracheal tube in the animal laboratory setting.

Effective pulmonary blood flow is calculated by integrating the difference in VCO_2 and p_ACO_2 between the rebreathing and non-rebreathing periods. The shunt fraction is calculated by incorporating estimated values of p_aO_2 derived from pulsoximetry and the inspired fraction of oxygen (F_iO_2) into Nunn's iso-shunt fraction plots (57). Cardiac output is obtained by adding the estimated shunt fraction to EPBF. There is an option to manually enter BL parameters, for example F_iO_2 and p_aO_2. This may contribute to an improved agreement when high F_iO_2 fractions are used (66).

The performance of the method has been compared to thermodilution in several animal and human studies. The results diverge from good to acceptable to poor agreement (59, 66-69). Lung injury with subsequent disturbances in the ventilation and perfusion ratio has been shown to negatively affect the performance of the method (67, 68). However, it should be noted that the majority of studies evaluate the NICO with the former version of software. A more recent study with updated software (5.0) found that the negative side effects of partial

rebreathing were reduced with preserved performance comparable with continuous CO measured by the PAC (69).

A capnodynamic equation for calculation of ELV and CO_{EPBF}

In 2013 Albu *et al.* described a novel mathematical approach to a capnodynamic equation for calculation of ELV (70). When evaluated in intubated and ventilated subjects the results were promising showing a high correlation between ELV and the reference method. The equation also includes a factor representing EPBF that was not evaluated *per se* in the study.

Without changing the tidal volume, alveolar ventilation was varied via multiple cyclic reoccurring short inspiratory pauses that resulted in alterations of the alveolar fraction of carbon dioxide (F_ACO_2) and elimination ($VTCO_2$) between breaths. These variations were integrated into the equation described below and the three unknown variables; ELV, EPBF and C_VCO_2 could be calculated.

$$ELV \cdot \left(F_A CO_2^n - F_A CO_2^{n-1} \right) = EPBF \cdot \Delta t^n \cdot \left(C_v CO_2 - C_c CO_2^n \right) - VTCO_2^n$$

The induced fluctuations in the alveolar concentration of carbon dioxide were approximately 4-8 mmHg. The equation compares the carbon dioxide content and the corresponding elimination induced by short periods of altered ventilation. After the prolonged breaths the ventilation frequency is slightly increased. This counteracts alterations of F_ACO_2 and abolishes the need for a period of equilibration. Therefore, the mixed venous content of carbon dioxide could be assumed to be constant and the breathing pattern and capnodynamic calculations could be performed continuously. In 2006 Peyton *et al.* suggested a similar approach for a semicontinuous capnodynamic method based on variations of the tidal volume (71).

In summary

Numerous studies evaluating cardiac output monitors *per se* or combined with a goal directed protocol for haemodynamic optimisation in relation to patient outcome have been published (2, 5, 17, 72, 73). Each monitor is associated with both advantages and disadvantages varying with the study population and haemodynamic conditions (3).

This thesis describes the first studies of a capnodynamic method in an animal model including significant haemodynamic and ventilatory challenges not possible to study in humans.

As clinicians we are exposed to new equipment not always properly evaluated but nevertheless introduced into the clinic (74). Our aim has been to stepwise challenge the method in extreme conditions to expose flaws and scrutinise the model. To accomplish this we have used a precise reference method considered to be the gold standard for CO monitoring in animal research (75, 76) and modern updated statistical methods including the

polar plot methodology (77). It has also been our intention to contribute with our medical expertise to further refinement and improvement of the method.

Maquet Critical Care AB, Solna, Sweden, developed the technical solution of the capnodynamic method evaluated it this thesis.

AIMS

The overall objective of this thesis was to evaluate a novel capnodynamic method for estimation of EPBF and ELV in a porcine model.

To validate CO_{EPBF} as assessed by a capnodynamic method based on inspiratory holds, against an ultrasonic reference method and CO_{PAC} during haemodynamic alterations.

To validate CO_{EPBF} as assessed by a capnodynamic method based on inspiratory holds against an ultrasonic reference method during haemodynamic and ventilatory alterations before and after lavaged-induced lung injury.

To evaluate the equation term, ELV, as assessed by a capnodynamic method based on inspiratory holds during CO alterations and the agreement and precision when compared to a reference method for FRC_{PEEP} during ventilatory alterations before and after lavaged-induced lung injury.

To validate CO_{EPBF} as assessed by a capnodynamic method based on *expiratory* holds against an ultrasonic reference method during haemodynamic and ventilatory alterations.

MATERIALS AND METHODS

Detailed methodological descriptions are given in each paper. General aspects are presented in the section below.

ETHICAL CONSIDERATIONS

The Animal Research Ethics Committee of Uppsala University approved of all experiments. The studies were performed at Hedenstierna Laboratory at Uppsala Academic University Hospital.

EXPERIMENTAL ANIMALS

In paper I ten pigs with a mean weight of 28 kg (range 24-29) were used. Papers II and III are based on data from ten pigs with mean weight of 35 kg (range 30-40). Eight pigs with mean weight of 34 kg (range 32-36) were used in paper IV. The experiments were conducted with the animals placed in the supine position except in paper I where they were positioned in the semilateral position.

ANAESTHESIA AND SURGICAL PREPARATION

Just after arrival to the laboratory, the animals were sedated via an intramuscular injection of 0.04 mg/kg atropine (NM Pharma AB, Sweden), 6 mg/kg tiletamine-zolazepam (Zoletil, Vibrac Laboratories, France), and 2.2 mg/kg xylazine chloride (Rompun, Bayer AG, Germany). After establishing an intravenous line a bolus dose of 5μg/kg fentanyl (Fentanyl B. Braun, Germany) was administered. The animals were intubated via direct laryngoscopy in paper I and via a tracheostomy in papers II-IV.

An intravenous infusion of ketamine 30 mg/kg/h, midazolam 0.1mg/kg/h and fentanyl 4 μg/kg/h was administered for maintenance of anaesthesia and rocuronium 2mg/kg/h for muscle relaxation. Anaesthetic level was evaluated prior to administration of muscle relaxant by applying pain stimuli to the fore hoof with a forceps.

Ventilation was performed in a volume-controlled mode (Servo-i; Maquet, Solna, Sweden) with a tidal volume of 10 mL/kg in papers I-III. In paper IV a tidal volume of 8 and 12 mL/kg was used. F_iO_2 was set to 0.4 in papers I and IV while in papers II and III, a F_iO_2 of 1.0 was used to calculate the true shunt fraction and to ensure animal stability during lung-lavage. Baseline positive end-expiratory pressure (PEEP) was set to 5 cmH_2O and the respiratory rate was adjusted to a frequency of 25 to 30 aiming at a systemic arterial partial pressure of carbon dioxide (p_aCO_2) of approximately 40 mmHg.

An intravenous infusion of Ringer's lactate was infused at the rate of 10 mL/kg/h. If there

were signs of hypovolemia, i.e. significant tachycardia and/or hypotension, additional colloid solution (Hesra, Baxter, Chicago, IL, USA) was administered in boluses of 100-200 mL.

The jugular vein and femoral artery were cannulated for administration of vasoactive drugs and arterial pressure recordings. An 8 Fr Fogarty occlusion catheter was inserted via ultrasound guidance into the femoral vein for preload reduction by intermittent occlusions of the caval vein. In papers I and IV a 13.5 Fr catheter was inserted into the femoral artery for controlled bleeding. A urinary catheter was placed via a small surgical incision into the urinary bladder and the body temperature was maintained at 38-39° C with warming mattresses and blankets.

When the preparation was finished the animals were left to recover for 30 minutes before BL recordings were obtained.

The animals were anaesthetised through the whole experiment and when it was completed, they were sacrificed by an injection of potassium chloride.

CARDIAC OUTPUT MONITORING

The ultrasonic flow probe

In all studies an ultrasonic flow probe (CO_{TS}) functioned as the reference method for CO and was placed around the pulmonary trunc via a left sided thoracotomy (T 401; Transonic system Inc, Ithaca NY, USA**)**. The size of the flow probe was adjusted to the size of the pulmonary trunk (16, 18 or 20 mm). Before the flow probe was put in position ultrasonic gel was applied between the vessel and the probe. The chest was then closed and the animals repositioned in the supine, papers II-IV or semilateral position in paper I (Figure 4).

Each CO_{TS} reading was based on an average of approximately ten seconds and recorded simultaneously with CO_{EPBF}. The inspiratory or expiratory holds within the nine or ten breaths cycle were randomly distributed in relation to the readings of CO_{TS}.

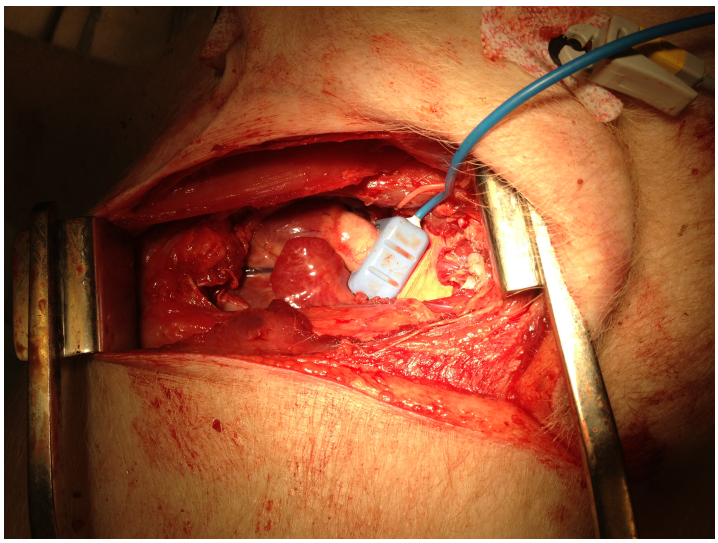

Figure 4. Via a left sided thoracotomy the pulmonary trunc was dissected. The size of the probe was selected by visual guidance. Ultrasonic gel was applied between the ultrasonic flow probe and the vessel.

The pulmonary artery catheter

A balloon-tipped 7.5 Fr PAC (CO_{PAC}) (Edwards Lifesciences, Irvine, CA, USA) was inserted via the right jugular vein into the pulmonary artery for monitoring of CO, pressure recordings and sampling of mixed venous blood gases. In paper I CO was determined by the mean of three 5 mL boluses of ice cold saline and in papers II-IV by the mean of three 10 mL boluses of ice cold saline. Because the injections caused instability of the carbon dioxide signal in the measured expired gas these measurements were always performed after the CO_{TS} and CO_{EPBF} readings.

Transpulmonary thermodilution

In paper IV a 4 Fr artery catheter (PiCCO Pulsion, Munich, Germany) was inserted via ultrasound guidance in the contralateral femoral vein and connected to the special device for the transpulmonary thermodilution $PICCO_2$ (PiCCO Pulsion, Munich, Germany). An average of three injections of 10 mL ice cold saline injected in the central venous catheter was used for calculation of CO at all interventions and at BL. The continuous pulse wave analysis was not used.

LUNG INTERVENTIONS AND MEASUREMENTS

Reference method for functional residual capacity

In paper III a device for dispensing the tracer gas, sulphur hexafluoride (SF6), was connected to the breathing circuit and SF6 was delivered in proportion to the inspiratory flow with a subsequent constant concentration regardless the inspiratory flow pattern. The amount of SF6 at the end of wash in was calculated during wash out and the functional residual capacity with a positive PEEP (FRC_{PEEP}) could be estimated (78).

Lung-lavage

In papers II and III lung injury (i.e. surfactant depletion) was induced by repeated lung-lavages (approximately 30 mL/kg) with 37º C saline solution at a F_iO_2 of 1.0 and PEEP 5 cmH_2O. The procedure was stopped after obtaining a stable P/F ratio of < 75.

Calculations of shunt and dead space

Berggren's formula was used for estimation of shunt fraction (79). Dead space was assessed by the classical Bohr's formula as described by Tusman *et al*. in paper II (80) and calculated according to Enghoff in paper III (81).

THE CAPNODYNAMIC METHOD

Effective pulmonary blood flow and ELV was calculated from a dynamic mole balance equation for the carbon dioxide content in the lung. Software in a modified ventilator (Maquet Critical Care, Solna, Sweden) induced the required changes by varying the ventilatory pattern in a predefined way. Data from the most recent ten or nine respiratory cycles are included in the on-going analysis.

The ventilatory pattern

In papers I-III the ten most recent breaths were included in the on-going analysis. Five subsequent respiratory cycles including a breath hold prolonging the inspiratory part of the cycle were followed by five normal respiratory cycles (Figure 5).

In paper IV the ventilatory pattern was modified and the number of breaths included in one test cycle was reduced to nine. Three respiratory cycles with an imposed end-expiratory pause were followed by six normal respiratory cycles (Figure 6).

To maintain an unaltered minute ventilation the breath rate for respiratory cycles without imposed pauses was increased.

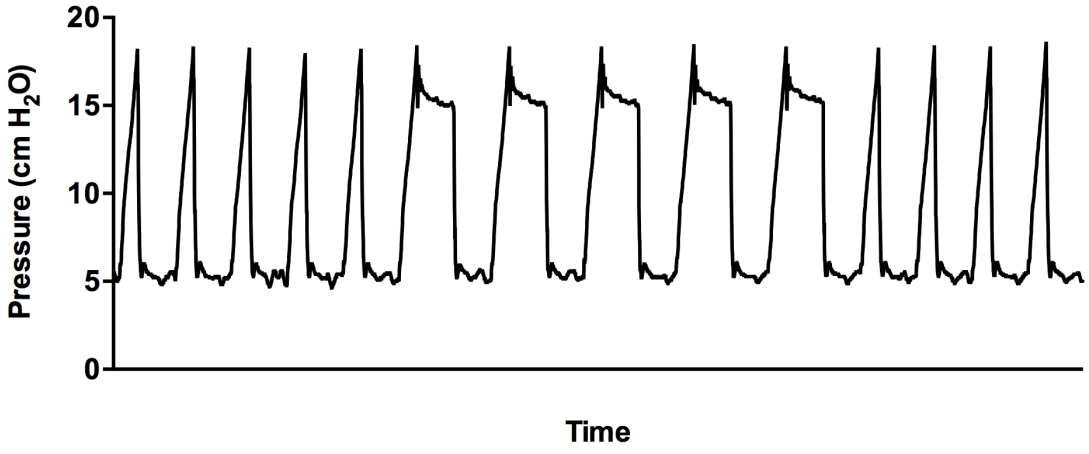

Figure 5. The ventilatory pattern in papers I-III, five normal cycles are followed by five respiratory cycles with an inspiratory hold.

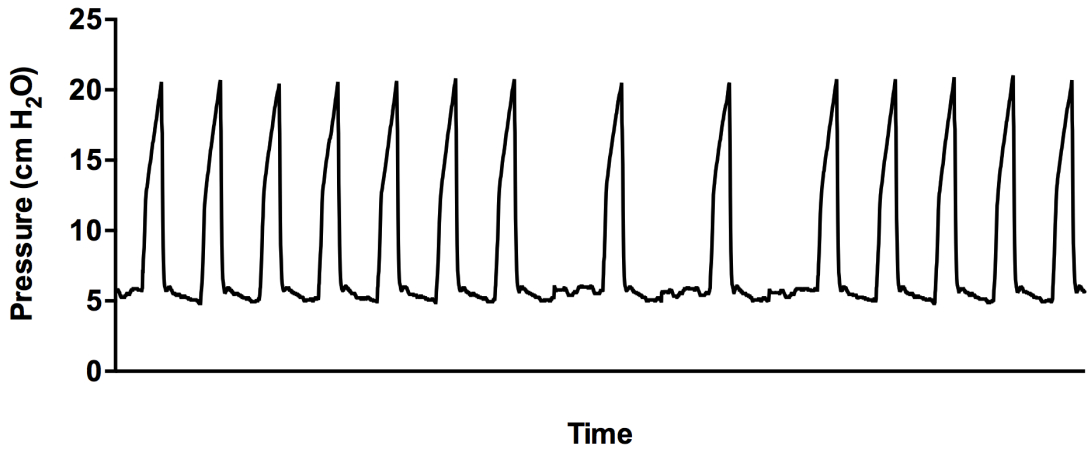

Figure 6. The ventilatory pattern in paper IV, six normal cycles are followed by three respiratory cycles with an expiratory hold.

The capnodynamic equation

The capnodynamic equation (see below) describes a mole balance of carbon dioxide between the transport of carbon dioxide to and from the lung and the rate of change of the carbon dioxide content in the lung induced by the alternating ventilatory pattern.

The left side of the equal sign expresses the difference in mean alveolar carbon dioxide content in the lung between two breaths (measured). Effective lung volume (calculated) represents the gas volume in the lung containing carbon dioxide.

The first term to the right of the equal sign, EPBF (calculated) represents the circulatory supply of carbon dioxide to the lung. Δt^{n} expresses the duration of each breathing cycle, which varies due to the imposed breath holds. C_vCO_2, venous carbon dioxide content (calculated) is assumed to be constant during a measurement cycle.

The carbon dioxide content of pulmonary end-capillary blood, $CcCO_2$, is calculated from the measured alveolar partial pressure, P_ACO_2, which is assumed to correlate closely to the partial pressure of carbon dioxide of the pulmonary end-capillary blood. To convert the partial pressure of carbon dioxide to concentration the impact of the dissociation curve for carbon dioxide in blood described by Capek *et al.* was used (63). The effect on the dissociation curve displayed by variation in the concentration of haemoglobin is included in the described formula and a haemoglobin value obtained at the beginning of the experiment was programmed into the software (63). The last term $VTCO_2^{n}$ displays the quantity of carbon dioxide exhaled by the n^{nt} tidal volume.

F_ACO_2 is estimated from the midpoint value of a linear function fit to the alveolar plateau (phase III) of the volumetric capnogram (82).

$$ELV \cdot \left(F_A CO_2^n - F_A CO_2^{n-1} \right) = EPBF \cdot \Delta t^n \cdot \left(C_v CO_2 - C_c CO_2^n \right) - VTCO_2^n$$

ELV	Effective lung volume [L]
EPBF	Effective pulmonary blood flow [L/min]
n	current breath
n-1	previous breath
F_ACO_2	mean alveolar carbon dioxide fraction
C_vCO_2	mixed venous carbon dioxide content [L_{gas}/L_{blood}]
$C_cCO_2^n$	end-pulmonary capillary carbon dioxide content [L_{gas}/L_{blood}]
$VTCO_2^n$	volume [L] of carbon dioxide eliminated by the current, n^{th}, breath
Δt^n	current breath cycle time [min]

Each breathing cycle results in a new equation. All quantities except ELV, EPBF and C_vCO_2 are directly determined from measurement breath-by-breath. An overdetermined set of ten or nine equations solved through a least-square-error optimisation of the fit between the left-hand-side and the right-hand-side of the equation calculates values for ELV, EPBF and C_vCO_2.

A prerequisite for this is the assumption that ELV, EPBF and C_vCO_2 remain constant during the complete measurement cycle consisting of a set of ten or nine breaths. The method is continuous because the measurement cycle is constantly updated with the most recent equation and expelling the last.

The measured data in the capnodynamic method are compared to an ideal one-lung compartment model. If the differences are too large between the optimal lung model and the measured data the equation cannot be solved and no values for ELV or EPBF can be calculated. A correction for the shunt fraction is not included.

DATA SAMPLING AND RECORDING

Pressure readings and haemodynamic signals were recorded and sampled (at 100 Hz) into a data acquisition system (Acknowledge, version 3.2.7, Bio Pac Systems, Santa Barbara, CA, US) to allow post processing of data.

Carbon dioxide and gas flow were measured by the ordinary mainstream carbon dioxide and flow sensors of the Servo-i ventilator, that were connected to a lap top computer where all the analysis were carried out by a software written in Matlab™ (Mathworks)

PAPER I

In paper I we evaluated CO_{EPBF} during significant haemodynamic alterations with CO_{TS} and CO_{PAC} as reference methods. The performance of CO_{PAC} was also evaluated *per se* with CO_{TS} as reference method. The ventilatory pattern was based on inspiratory holds.

Experimental protocol

After BL measurements were recorded the following interventions were performed:

(Figure 7)

1) Preload reduction by partial caval occlusion reducing CO_{TS} by 50%.
2) Afterload increase by infusion of phenylephrine increasing mean arterial pressure (MAP) to 150% of BL.
3) Decreasing afterload by an infusion of nitroprusside decreasing MAP to 60% of BL.
4) Inotropic stimulation by infusion of dobutamine increasing CO_{TS} to 200% of BL.
5) An intravenous volume challenge of 500 mL colloid.
6) Controlled bleeding reduced MAP to 35 mmHg.

Each haemodynamic intervention was followed by a stabilisation period where one BL reading was performed. At initial BL and during all haemodynamic interventions three readings were obtained except during haemorrhage were two readings were performed.

All paired CO data were recorded under haemodynamic steady state conditions as judged by the CO_{TS}.

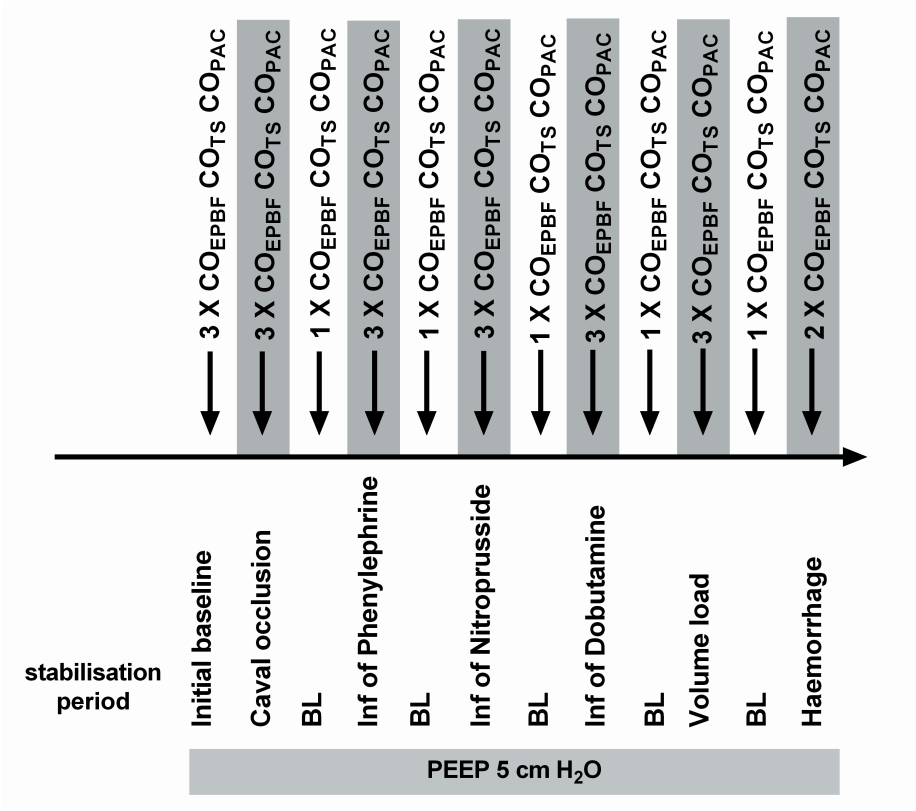

Figure 7. A schematic description of the haemodynamic interventions in paper I. Three consecutive measurements by all methods were obtained at initial baseline and interventions expect during haemorrhage where two measurements were performed. At baseline between interventions, one measurement was obtained. A PEEP of 5 cm H_2O was used during the whole experiment (N=10).

PAPER II

In paper II we evaluated CO_{EPBF} during significant haemodynamic and PEEP alterations with CO_{TS} as the reference method before and after lung-lavage. The ventilatory pattern was based on inspiratory holds.

Experimental protocol

After BL measurements were recorded the animals were randomised to start the haemodynamic interventions at PEEP 5 or 12 cmH_2O. Then the haemodynamic situation was altered by:

(Figure 8)

1) Preload reduction by caval occlusion aiming to decrease CO_{TS} to 50% of BL.
2) Infusion of dobutamine titrated to increase CO_{TS} to 150% of BL.

The PEEP level was then altered and the haemodynamic interventions repeated in the same order.

After lung-lavage, described above, the haemodynamic interventions were repeated first at PEEP 5 cmH_2O and then at PEEP 12 cmH_2O.

Between all interventions there was a recovery period at BL conditions where measurements were obtained. During each intervention and at BL two paired CO measurements were obtained in haemodynamic steady state conditions as judged by CO_{TS}.

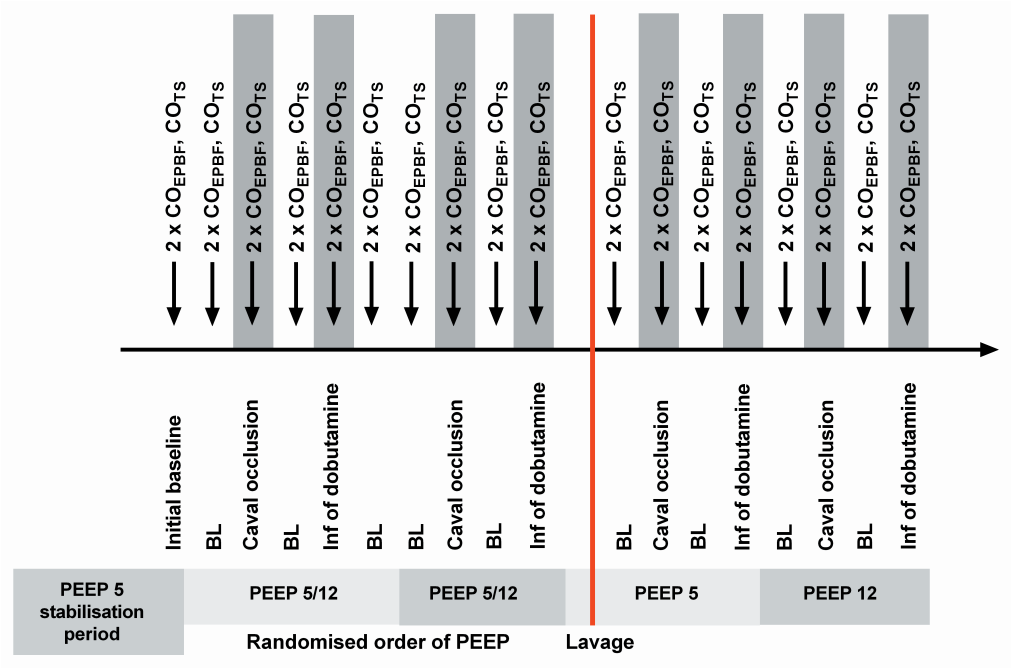

Figure 8. A schematic description of the protocolled interventions in paper II. After the initial baseline (BL) measurements, the animals were randomised to start at PEEP 5 or 12 cmH_2O. At each PEEP step the same sequence including BL, preload reduction and inotropic stimulation was induced and two measurements were obtained at each intervention and BL. The red line represents lung-lavage. The protocol was then repeated after lavage, but not in a randomised PEEP order (N=9).

PAPER III

In paper III we assessed the stability of ELV during significant CO alterations and compared ELV to a reference method for FRC at different PEEP levels before and after lavage. The ventilatory pattern was based on inspiratory holds.

Experimental protocol

After BL measurements were recorded the animals were randomised to start the haemodynamic interventions at PEEP 5 or 12 cmH_2O. Then the haemodynamic situation was altered by:

(Figure 9)

1) Preload reduction by caval occlusion aiming to a reduction of CO_{TS} to 50% of BL.
2) Infusion of dobutamine titrated to increase CO_{TS} to 150% of BL.

The PEEP level was then altered and the haemodynamic interventions repeated in the same order.

After lung-lavage, described above, the haemodynamic interventions were repeated first at PEEP 5 cmH_2O and then at PEEP 12 cmH_2O. Before ending the experiment the PEEP level was reduced to 0 where thereafter CO, ELV, and FRC_{PEEP} were recorded (N=7).

Effective lung volume was recorded at BL and during all interventions. Due to haemodynamic instability during preload reduction and inotropic stimulation the corresponding FRC_{PEEP} measurements were obtained only at BL.

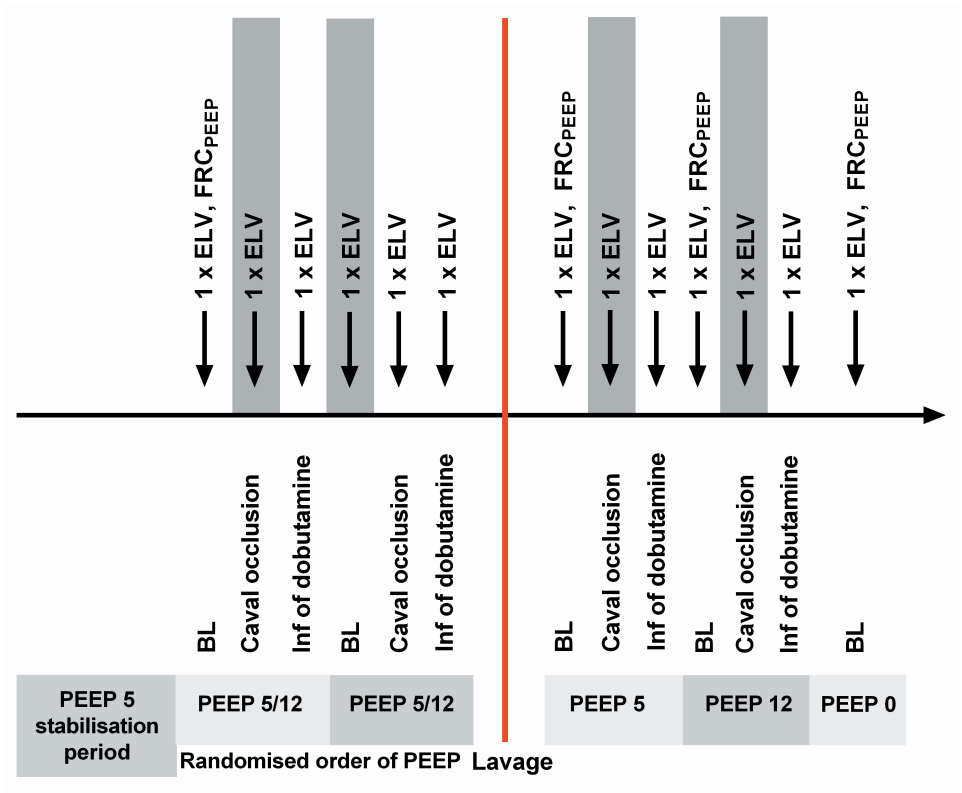

Figure 9. A schematic description of the protocolled interventions in paper III. After the initial baseline measurements, not included in the graph, the animals were randomised to start at PEEP 5 or 12 cmH_2O. Baseline measurements were followed by preload reduction and inotropic stimulation. The PEEP level was altered and the interventions repeated. After lung-lavage was performed, represented by the red line, the interventions were repeated but not in a randomised order. ELV (mL) values were obtained during baseline and interventions. Due to haemodynamic instability FRC_{PEEP} (mL) was measured only at baseline conditions (N=7-9).

PAPER IV

In paper IV we evaluated CO_{EPBF} during haemodynamic and PEEP alterations as well as different tidal volumes with CO_{TS} as the reference method. CO_{PAC} and CO_{TPT} were also compared to CO_{TS} *per se.* The ventilatory pattern was based on *expiratory holds*.

Experimental protocol

Baseline measurements of CO_{TS}, CO_{EPBF}, CO_{PAC} and CO_{TPT} were obtained at PEEP 5 cm H_2O and a TV of 8 mL/kg. The haemodynamic situation and ventilator settings were then altered by:

(Figure 10)

1) PEEP 0 cmH_2O and 12 cmH_2O
2) Increased tidal volume of 50% from 8 to 12 mL/kg
3) At BL conditions preload reduction to CO_{TS} 50% of BL by caval balloon inflation.
4) Increase of PEEP from 5 to 12 cmH_2O
5) Preload reduction of CO_{TS} 50% of BL at PEEP 12 cmH_2O by caval balloon inflation.
6) Alteration of the PEEP level from 12 to 5 cmH_2O.
7) At BL conditions increase of CO_{TS} to 150% by an infusion of dobutamine
8) Controlled bleeding titrated to a MAP of 35 mmHg.

All measurements were accomplished at steady state conditions as judged by CO_{TS}. During all interventions one measurement was obtained except at preload reduction PEEP 5 and 12 cmH_2O, inotropic stimulation and haemorrhage where two measurements were recorded. Between all interventions there was a recovery period at BL conditions where one measurement was obtained.

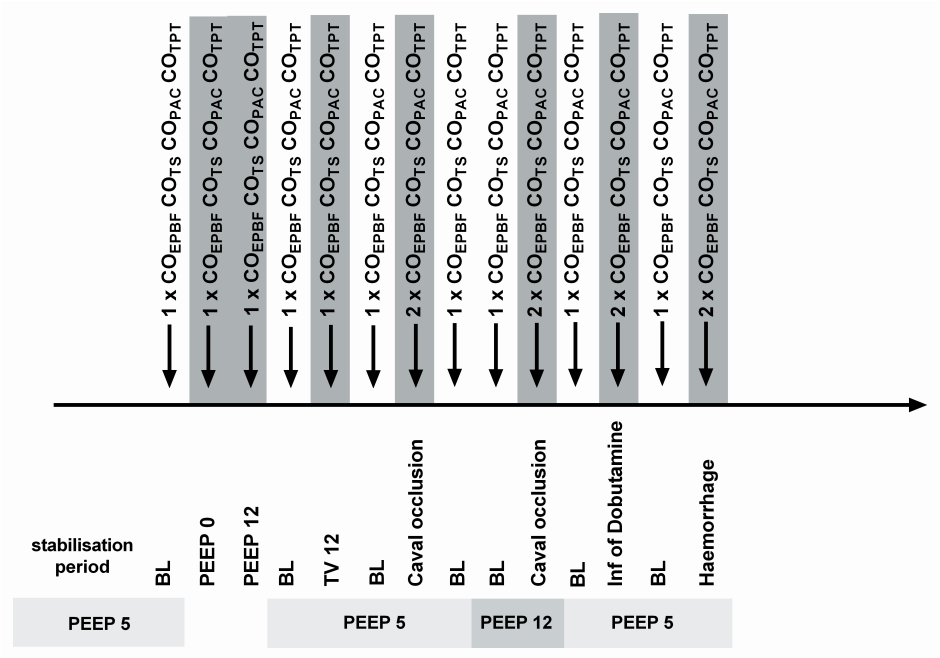

Figure 10. A schematic description of the haemodynamic and ventilatory manipulations in paper IV. After a stabilisation period and baseline measurements the ventilatory settings were changed by alterations of PEEP and tidal volume (TV). This was followed by haemodynamic alterations at two different PEEP levels. One measurement from each monitor was obtained at baselines and all interventions except during preload reduction, inotropic stimulation and haemorrhage where two measurements were obtained. (N=8).

STATISTICAL METHODS AND CALCULATIONS

Cardiac output data were checked for normal distribution by the Kolmogorov–Smirnov test in paper I and D'Agostino and Pearson omnibus K2 test in papers II-IV. Wilcoxon's matched-pairs signed rank test was used to calculate significant differences in paper II. A

one-way ANOVA for repeated measurements was used for calculation of differences in ELV between the corresponding PEEP levels before and after lavage in paper III. A paired t-test was used for calculations of p-values between ELV and FRC_{PEEP} within each PEEP step. Pearson's correlation coefficient was used to assess the correlation between absolute values and paired delta values. A p -value of < 0.05 was considered statistical significant.

Inherent precision

Inherent precision was defined as twice the coefficient of variation (CV). CV= SD/mean (83).

In paper I the inherent precision for CO_{EPBF} and CO_{TS} were calculated across all animals by including three consecutive BL measurements from each CO monitor during haemodynamic steady state. The inherent precision of CO_{PAC} was calculated as twice the coefficient of error (CE) (CV/√3), of the three averaged thermodilution curves from the same BL period.

In papers II and IV CO_{EPBF} data and in paper III ELV data from each tidal volume was collected from the sampling system, typically during 5-10 minutes, at the first BL period. Standard deviation of the included CO data and mean CO were calculated across all animals. The inherent precisions of CO_{EPBF} and ELV were recalculated at BL conditions after lung-lavage was performed in papers II and III.

Agreement

Bland Altman statistics were used for evaluation of absolute values. (84). When repeated measurements were obtained from the same subject an adjustment for multiple measurements was made (85).

Trueness defined as the mean difference between the test and the reference method was described by bias (84, 86). Limits of agreement (LoA) estimate the spread of the included paired values and were calculated as bias ± (1.96xSD). 95% of the paired data will be positioned within those limits (84, 86) (Figure 11).

The percentage error (PE), relating the spread in the sample to the mean of the reference method, was calculated as 1.96xSD divided by the mean of the reference method (84). *A priori* we considered a PE $< 30\%$ as an indication of the two methods being interchangeable and $< 45\%$ as a limit of acceptance when thermodilution was used as a reference method for CO (83, 87, 88).

Confidence intervals (95%) were calculated for bias, LoA and the mean polar angle in papers II and IV.

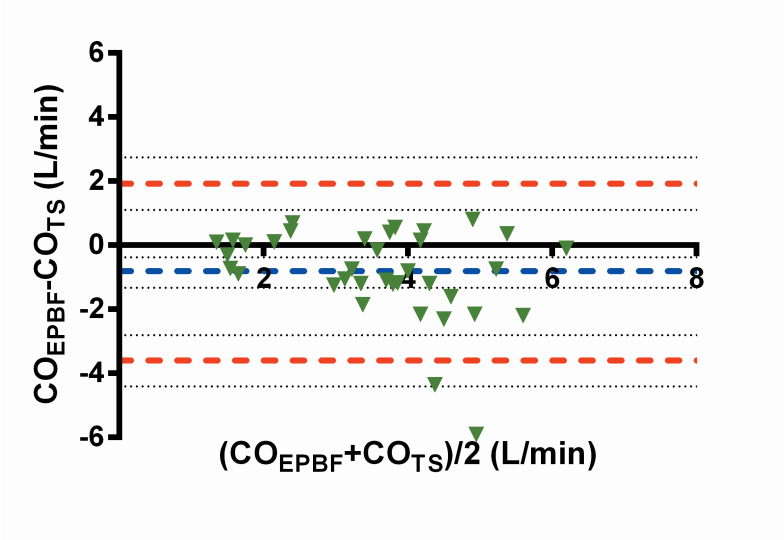

Figure 11. An example of a Bland Altman plot (paper II) assessing the agreement for absolute values. The green triangles represent 36 paired values displaying the difference between the capnodynamic (CO_{EPBF}) and the reference method for cardiac output (CO_{TS}) plotted against the mean of the corresponding data at PEEP 5 cmH_2O after lung-lavage. The dotted blue line represents bias (L/min), i.e. the mean difference between the two methods. The red dotted lines represent limits of agreement (LoA) (L/min) defining the area that includes 95% of all the individual bias values. The black dotted lines represent confidence intervals of bias and LoA.

Trending ability

The agreement for the paired delta values was evaluated by two different methodologies assessing the concordance rate, the four-quadrant (4Q) (89) and the polar (PP) methodology (77). The 4Q methodology estimates the direction of change calculating the concordance rate as the number of paired delta values positioned in one of the two concordant quadrants divided by the total number of paired delta values (89). (Figure 12) The PP methodology also includes the magnitude of change in the concordance rate and calibration between methods by calculation of the mean polar angle (angular bias). Concordance rate was calculated as the number of paired delta values within the radial limits of agreement of ± 30° divided by the total number of paired delta values (77) (Figure 13).

Since central data points reflect random measurement errors rather than trending ability an exclusion zone of 10-15% was used for both methods (77, 89, 90).

A concordance rate of < 90% was considered as poor, > 90-95% as acceptable to good for the 4Q methodology (89, 91). A concordance rate of > 90% was considered acceptable for the PP methodology (91). An angular bias of equal or less then ±5° indicated good calibration of the test method in comparison with the reference method (77).

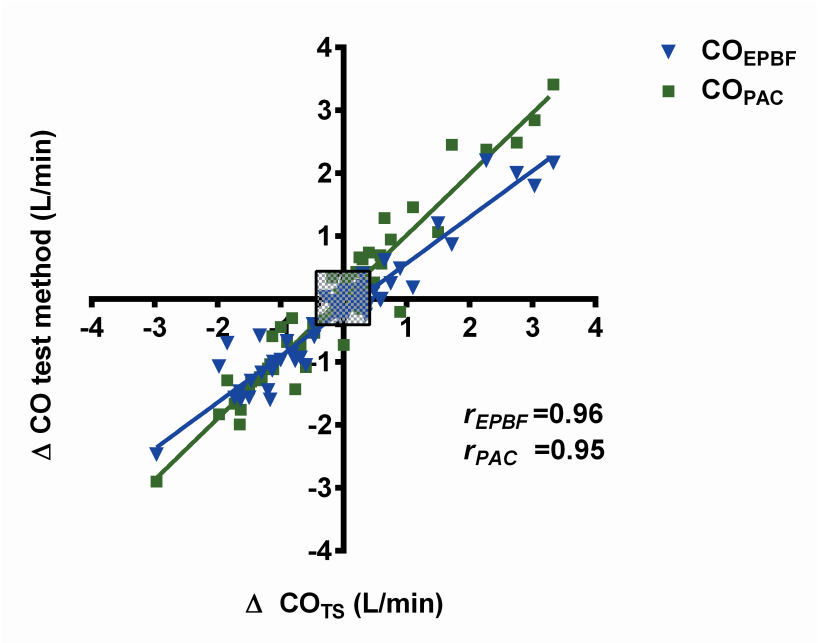

Figure 12. An example of a four-quadrant plot from paper I showing the trending abilities for the capnodynamic method (CO_{EPBF}) blue triangles and thermodilution (CO_{PAC}) green squares compared to the reference method for CO (CO_{TS}). Fifty-six paired delta CO values (L/min) are displayed. The correlation coefficients for both CO_{EPBF} and CO_{PAC} are also shown. Data points plotted in the upper right and lower left quadrants are considered concordant. The shadowed square in the middle marks the exclusion zone, in this example 15% (0.4 L/min), corresponding to small changes in CO considered to reflect random measurement errors rather than trending ability.

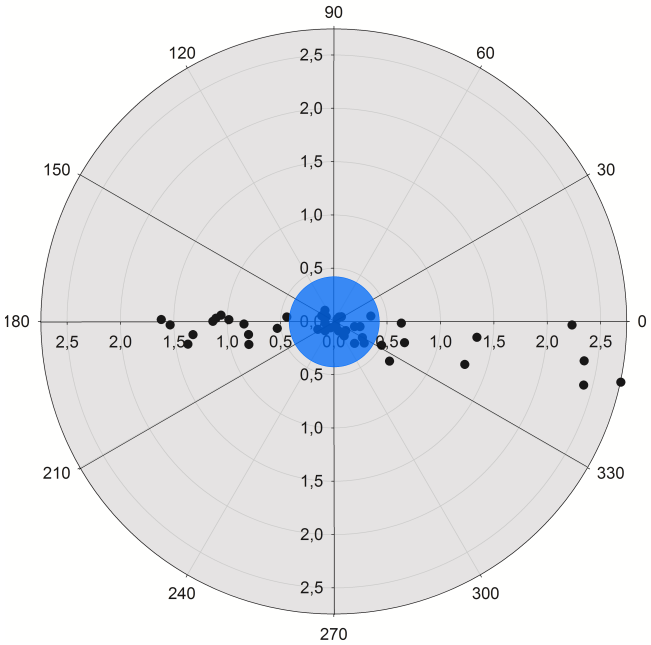

Figure 13. An example of a polar plot from paper I for the capnodynamic method (CO_{EPBF}) using the ultrasonic flow probe CO_{TS} as reference method. The blue zone in the middle represents the exclusion zone. Data spread closely to the polar axis indicates good trending. Lines indicate ±30° radial limits.

Statistical computer programs

All statistical calculations except for the polar plots were done in GraphPad Prism (version 6.0 for Windows, GraphPad Software, San Diego, CA, USA). For calculation of polar plots, an excel sheet for conversion of Cartesian data to polar coordinates was used kindly provided by Professor L Critchley.

Sigma Plot (version 12.0 for Windows, Systat software, Inc., GmbH, Germany) was used to construct the polar plots.

RESULTS

All animals survived the protocolled interventions. Data from one animal were excluded because of problems with data acquisition in papers II and III.

PAPER I

The haemodynamic interventions resulted in marked alterations of CO_{TS} ranging from 1.2 to 4.9 (mean 2.6) L/min (Figure 14).

Inherent precision was calculated for all CO monitors: CO_{TS} ± 1%, CO_{EPBF} ± 2.5%, and CO_{PAC} ± 4.3% at BL. The inherent precision of CO_{PAC} was ± 7%.

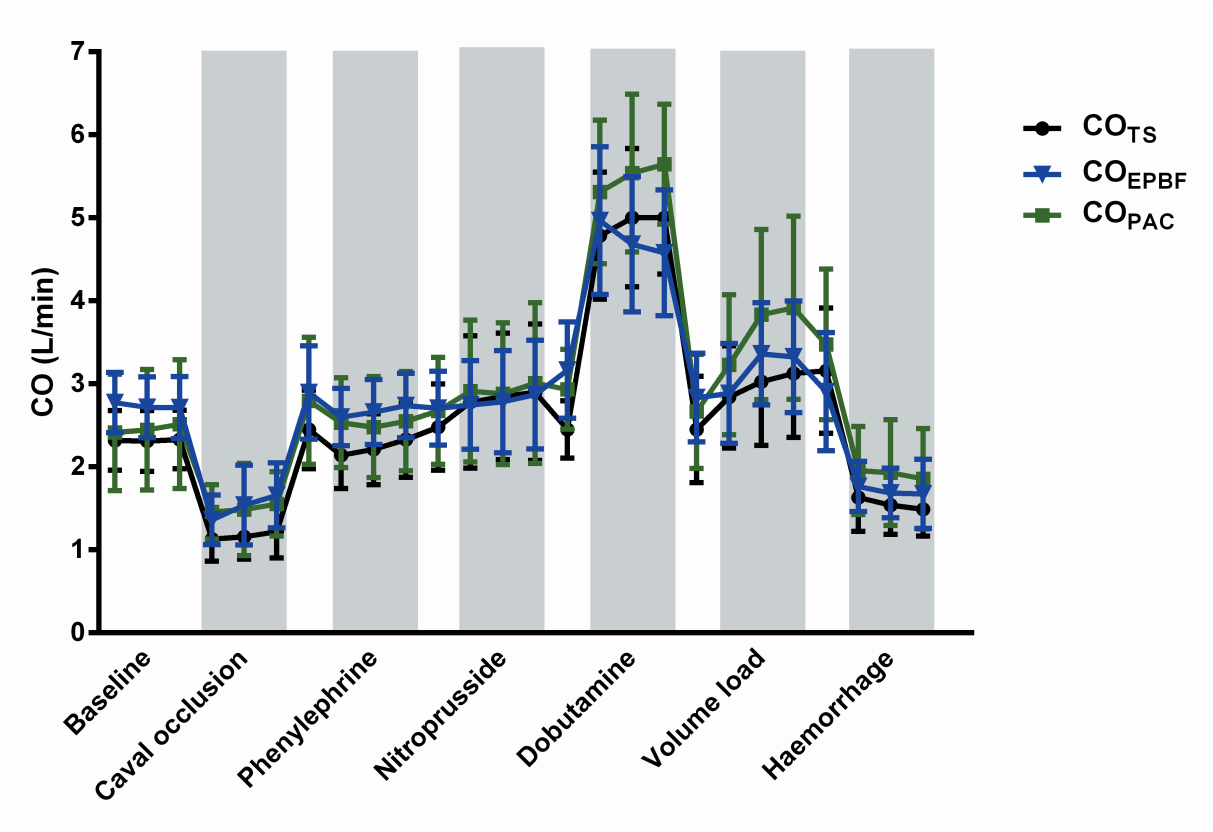

Figure 14. Cardiac output (L/min) obtained by the reference method for CO (CO_{TS}) black dots, the capnodynamic method (CO_{EPBF}) blue triangles, and the pulmonary artery catheter (CO_{PAC}) green squares at baseline and during haemodynamic interventions. Each haemodynamic challenge was followed by a baseline stabilisation period. Data are presented as mean (±SD) (N=6-10).

Agreement

CO_{EPBF} and CO_{PAC} showed an overall consistency in relation to CO_{TS} in response to the haemodynamic interventions. CO_{EPBF} tended to overestimate CO slightly with an overall bias (LoA) of 0.2 (-1.0 to1.4) L/min. This result was comparable to the corresponding values for CO_{PAC} showing similarly good agreement with bias (LoA) of 0.3 (-1.0 to1.6) L/min. However, the overall percentage error was 47% for CO_{EPBF} and 49% for CO_{PAC}.

CO_{EPBF} was also compared to CO_{PAC}, being a clinical reference method for CO. CO_{EPBF} showed good agreement for all interventions except during dobutamine infusion when CO_{EPBF} underestimated and CO_{PAC} overestimated CO as assessed by CO_{TS}.

Trending ability

The concordance rate, as assessed by the four-quadrant methodology was 97% and 95% for CO_{EPBF} and CO_{PAC} respectively. When the polar plot methodology was used, the corresponding values were 97% (angular bias of -5.6°) and 100% (angular bias of 0.93) for CO_{EPBF} and CO_{PAC} respectively.

PAPER II

The haemodynamic interventions resulted in marked alterations of CO_{TS} ranging from 0.9-8.9 (mean 3.4) L/min (Figure 15).

Inherent precision was calculated for CO_{EPBF} and remained ± 9% both before and after lung-lavage. The inherent precision of CO_{TS} was previously shown to be ± 10% (92).

Lung-lavage resulted in a significant decrease in lung function with a two-fold increase in shunt fraction and decreased compliance. When PEEP was increased to 12 cmH_2O after lung-lavage, the shunt fraction was reduced indicating a restored lung function.

At each PEEP level the shunt fraction was dependent of CO regardless of the degree of lung injury and PEEP level. The difference between CO_{EPBF} and CO_{TS} increased with increased shunt flow. When shunt levels was > 20%, CO_{EPBF} underestimated CO_{TS}.

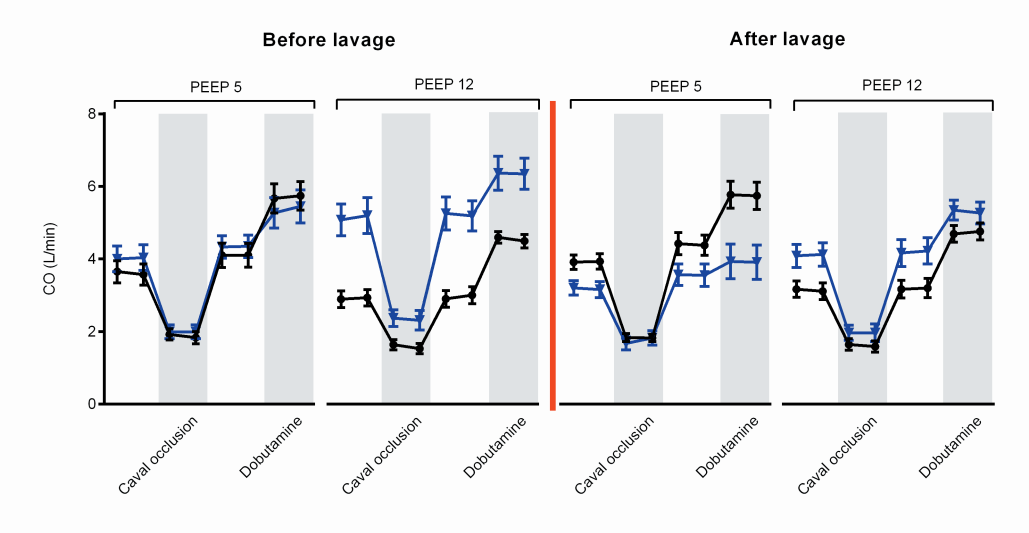

Figure 15. Cardiac output (L/min) displayed by the reference method for cardiac output (CO_{TS}) black dots and the capnodynamic method (CO_{EPBF}) blue triangles at baseline and during haemodynamic interventions at PEEP 5 and 12 cmH_2O before and after lung-lavage. Data are presented as mean (± SD). The red vertical line represents lung-lavage (N = 9).

Agreement

When PEEP was increased to 12 cm H_2O before lung-lavage CO_{EPBF} showed a paradoxical increase compared to CO_{TS} bias (LoA) and PE 1.4 (-1.3 to 4.2) l/min and 90% (table). Lung-lavage affected the performance of CO_{EPBF} resulting in decreased agreement and precision with bias (LoA) and PE of -0.9 (-3.6 to 1.9) 70% (Table 1).

Trending ability

The trending ability was preserved before and after lung-lavage with a concordance rate of 94% as assessed by the four-quadrant methodology. The polar plot methodology showed a concordance rate of 100% and an angular bias of -4.0° at PEEP 5 cm H_2O before lung-lavage. However, after lavage the concordance rate decreased to 89% and the angular bias was -16.4° indicating suboptimal calibration (Table 1).

	PEEP 5 (cmH_2O) before lavage	PEEP 12 (cmH_2O) before lavage	PEEP5 (cmH_2O) after lavage	PEEP 12 (cmH_2O) after lavage
Bias (LoA) PE	0.2 (-1.1 to 1.5) 34% n=45	1.4 (-1.3 to 4.2) 90% n=36	-0.9 (-3.6 to 1.9) 70% n=36	0.8 (-1.5 to 3.1) 75% n=36
4Q	94%	94%	94%	100%
PP	100% -4.0°	89% 3.1°	89% -16.4°	89% -2.5°

Table I. Paper II, agreement between the capnodynamic method (CO_{EPBF}) and the reference method for CO (CO_{TS}) as assessed by the Bland Altman methodology. Bias, limits of agreement (LoA) (L/min) and percentage error (PE %) are displayed for the different PEEP levels before and after lung-lavage. Two different methodologies were used to assess the trending ability (concordance %), the four-quadrant (4Q) and the polar (PP) methodology. The mean polar angle indicates how well the test method is calibrated to the reference method. Eighteen paired delta values were included in calculation of concordance rate (N=9).

PAPER III

The haemodynamic interventions resulted in marked alterations of CO_{TS} ranging from 0.9-8.9 (mean 3.4) L/min.

Inherent precision for ELV was calculated both before and after lavage to ± 4.5% and ± 9.0 respectively. The inherent precision of CO_{TS} was previously shown to be ± 10% (92) and the corresponding value for FRC_{PEEP} was ± 6.0% (78).

Lung-lavage caused a significant increase in shunt fraction and a 40% reduction of FRC_{PEEP} indicating deterioration of the lung function.

ELV was not significantly affected by CO alterations within the same PEEP level. A small difference between ELV and FRC_{PEEP} was seen at PEEP 5 cmH_2O before lavage and at PEEP 12 cmH_2O after lavage. (Figure 16)

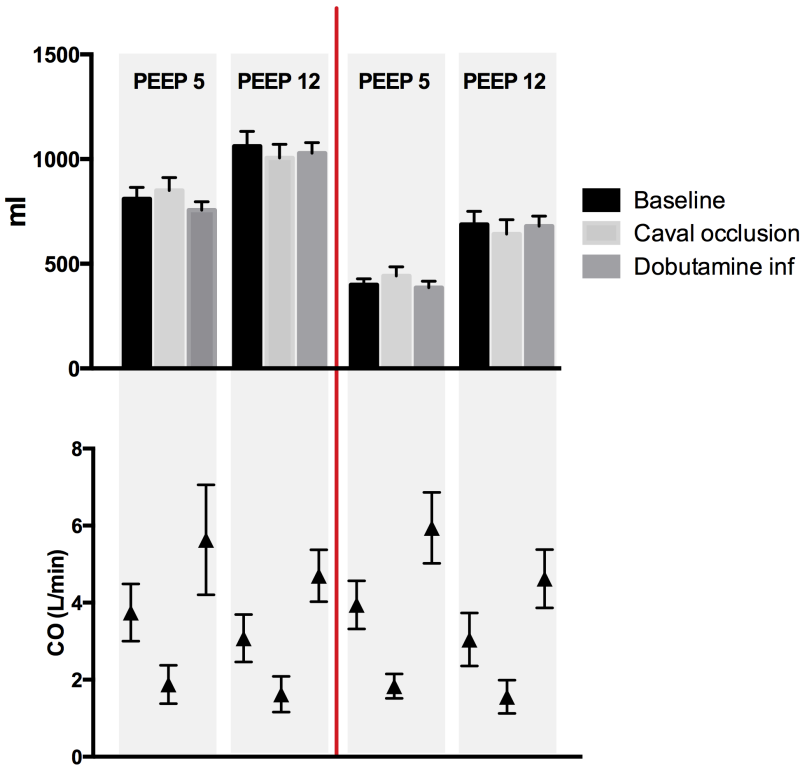

Figure 16. Upper panel shows the effective lung volume, ELV (mL) during caval occlusion and dobutamine infusion at different PEEP levels before and after lavage. Lower panel displays the corresponding cardiac output values (L/min), obtained by the reference method for CO. Data are displayed as mean (± SEM). No significant differences in ELV were seen within the PEEP steps using one-way ANOVA analysis. The red line represents lung-lavage (N=9).

A high shunt fraction impaired the agreement between ELV and FRC_{PEEP} (Figure 17).

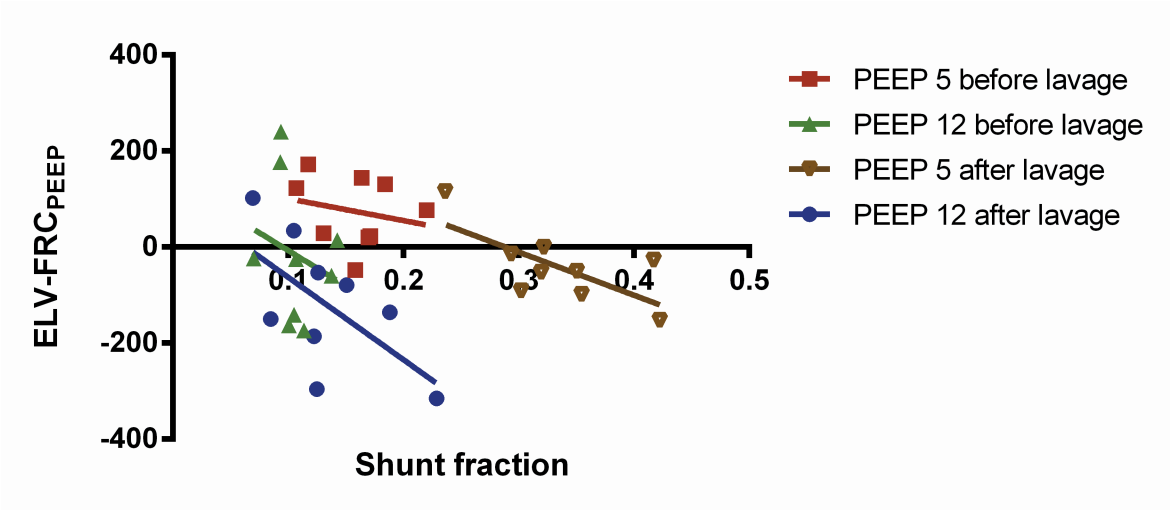

Figure 17. The difference between ELV (mL) and the reference method for FRC, FRC_{PEEP} (mL), plotted against shunt fraction. The deviation from zero is significant in PEEP 5 after lung-lavage (p=0.03).

Agreement

The overall agreement was, bias (LoA) and PE, -35 (-271 to 201) mL, and 36%. When data were divided in two groups, before and after lavage the corresponding values was 29 (-207 to 263) mL, 26% and -77 (-272 to 118) mL, 39%, respectively.

Trending ability

The ability for ELV to detect trends between PEEP steps, showed a concordance rate of 100% as assessed by the four-quadrant methodology.

PAPER IV

The haemodynamic interventions resulted in marked alterations of CO_{TS} ranging from 1.3 and 8.0 (mean 3.2) L/min (Figure 18).

Inherent precision for CO_{EPBF} was calculated to ± 14%. The inherent precision of CO_{TS} was preciously shown to be ± 10% (92) and ± 8% for CO_{TPT} (47).

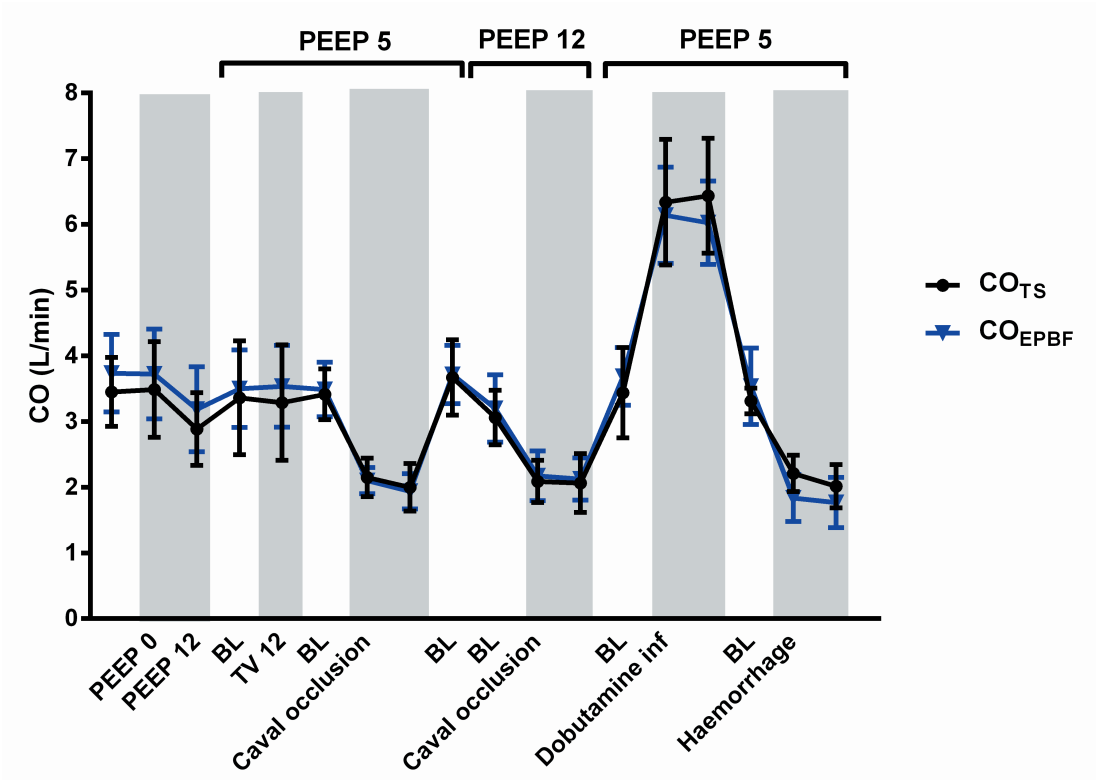

Figure 18. Cardiac output (L/min) is displayed on the y-axis as measured by the reference method for CO (CO_{TS}), black dots and the capnodynamic method (CO_{EPBF}) blue triangles. The haemodynamic and ventilatory interventions are displayed on the x-axis. The corresponding PEEP values are displayed at the top of the diagram (cmH_2O). Data are presented as mean (±SD) (N=8).

Agreement

The overall agreement for CO_{EPBF} was preserved compared to the results in paper I and the precision was improved with an overall PE of 36%. At high PEEP levels a decrease in precision was seen with a PE of 41% and wider LoA. The overall performance of CO_{PAC} was equal compared to our previous results in paper I.

CO_{TPT} displayed the best precision of all methods with a PE < 30% in all intervention steps except during inotropic stimulation. Data for absolute values of the three different methods are displayed in table 2.

Trending ability

Sixty-two paired delta values of CO_{EPBF} and CO_{TS} were analysed by the four-quadrant and the polar plot methodology, concordance rate was 97% and 94% respectively (mean polar angle 0.4°).

The corresponding values for CO_{PAC} was 96% and 92% (mean polar angle -8.1°) and CO_{TPT} 97% and 95% (mean polar angle 1.8°) (58 paired delta values).

	CO_{EPBF} bias (LoA) PE	**CO_{PAC} bias (LoA) PE**	**CO_{TPT} bias (LoA) PE**
Overall	0.05 (-1.1-1.2) 36% (n=141)	-0.2 (-1.6-1.2) 45% (n=97)	0.5 (-0.4-1.5) 29% (n=109)
PEEP 5	0.1 (-0.9-1.2) 32% (n=70)	-0.2 (-1.7-1.2) 38% (n=48)	0.5 (0.6-1.6) 29% (n=54)
PEEP 12	0.2(1.0-1.4) 41% (n=32)	0.1 (-1.3-0.1) 46% (n=21)	-0.5 (-0.3-1.2) 30% (n=24)
High CO	-0.3 (-2-1.4) 27% (n=16)	0.09 (-1.4-1.6) 24% (n=7)	-0.05 (-2.1-2) 31% (n=14)
Low CO	-0.09 (-0.8-0.6) 33% (n=46)	-0.08 (-1.0-0.8) 45% (n=21)	0.43 (-0.2-1.1) 28% (n=24)

Table 2. Table showing results from Bland Altman analysis for the capnodynamic method (CO_{EPBF}), the pulmonary

artery catheter (CO_{PAC}) and the transpulmonary thermodilution (CO_{TPT}) when compared to the ultrasonic flow probe (CO_{TS}) (L/min). The paired measurements are separated into five subgroups; overall, including all measurements, the two different PEEP steps 5 and 12 cmH_2O, high (inotropic stimulation) and low (preload reduction and haemorrhage) CO states. The bias, limits of agreement (LoA) (L/min) and percentage error (PE %) for each method are displayed in the corresponding column. n=numbers of paired CO values included in the calculation (N=8).

DISCUSSION

In this thesis we have evaluated and further developed a capnodynamic method for assessment of EPBF and ELV. We used significant haemodynamic and ventilatory alterations in order to scrutinise the method during extreme conditions. Moreover, we used modern statistical methods. We have found that the overall agreement and trending ability were comparable to the invasive clinical reference method for CO and that ELV remained stable during haemodynamic alterations.

However, lung-lavage with subsequent surfactant depletion impaired the performance of CO_{EPBF} especially when the shunt fraction was over 20% leading to an underestimation of CO. The paradoxical increase of CO_{EPBF} when PEEP was increased to 12 cmH_2O in paper II was not seen when the CO_{EPBF} was evaluated with the expiratory-based ventilation pattern. Furthermore, the overall precision was improved when using the modified ventilator setting.

CHOICE OF ANIMAL MODEL AND REFERENCE METHODS

Pigs are in the physiological sense rather similar to humans even though there are apparent differences (93, 94). Because of the requirement for extensive surgical procedures, instrumentation and frequent blood sampling, the size of the animals also had to be suitable. In paper I the animals were smaller compared to the animals in the following papers. This made the preparation more difficult and the lower BL CO could have negatively affected some of the statistical calculations, such as PE (83). Since the method was evaluated for potential use in adults and the experimental model required significant manipulations larger sized pigs were selected for papers II-IV.

Our decision to use multiple lung-lavages to induce surfactant depletion may be questioned since this model induces a non-sustainable decrease in lung function more than a persistent lung-injury (95). However, our intention was not to study lung injury *per se* but to study how the performance of CO_{EPBF} was affected by this temporary disturbance in lung function with subsequent increased shunt fractions and low P/F ratio. Furthermore, the lavage model is reproducible and easy to perform. It is also well tolerated by the animals since it is performed in a stepwise way and could be adjusted to the individual response.

Limitations also include the choice of reference methods. CO_{TS} measures flow in the pulmonary trunk with high agreement and precision (75, 76). CO_{EPBF} on the other hand estimates non-shunted blood flow. In summary, the test and the reference method do not measure the exact same entity. However, since there is a lack of a reference method for EPBF, we decided to use CO_{TS} considering it is the gold standard for flow measurements in animal research. Furthermore, EBPF could be assumed to correlate closely to CO when shunt fractions are low.

Also the relationship between ELV and FRC_{PEEP} is complicated since they measure slightly different lung volumes. FRC_{PEEP} measures an anatomical lung volume where the tracer gas

spreads in all open parts of the alveoli whereas ELV corresponds to the functional lung volume participating in carbon dioxide exchange. The SF6 has been found to correlate with Helium wash in/wash out, a reference method for FRC (78). Furthermore, the measurements are reproducible and easy to obtain and the method could be used when PEEP is applied.

INHERENT PRECISION

We calculated the inherent precision of CO_{EPBF} in all papers. In paper I the precision was high, a finding that was not repeated in the following papers. A difference in the methodology when data were sampled could have contributed to this finding. In paper I we included three BL values for each monitor. In papers II-IV we improved our methodology and included longer BL periods collected from the data sampling system and analysed the SD and mean across all included values for calculation of precision. This approach when multiple values were included in the analysis resulted in lower inherent precision in all papers. However, the inherent precision was still under 20%.

TRENDING ABILITY

Interest in functional haemodynamic monitoring is increasing and the advantages of monitoring CO trends have been highlighted in the literature (2, 17, 83). Several publications argue that the ability to rapidly detect trends is more important than the agreement of absolute values when goal-directed protocols are used (72, 96).

When trending ability was assessed by the traditional 4Q methodology, concordance rates were acceptable or good. However, when the modern PP methodology also assessing the magnitude of the changes was used, concordance rate decreased during high PEEP levels and after surfactant depletion.

In multiple studies CO_{TS} displays an inherent precision below 20% (75, 76). Critchley *et al.* suggests that the exclusion zones should be narrower when the inherent precision of the reference method is lower than 20% (90). In paper I we used an exclusion zone of 15%. Considering the low precision of CO_{TS} this limit could be considered too generous. In papers II-IV we adjusted the exclusion zones to 10%.

AGREEMENT AND PRECISION

CO_{EPBF} performed equally when compared to the clinical reference method CO_{PAC} regarding overall agreement. When compared to the more precise invasive method, CO_{TS}, bias was considered low in all comparisons except in paper II at high PEEP levels and after surfactant depletion.

A definition of an acceptable limit for bias is lacking in the literature. It is therefore left to the authors to decide what is acceptable in the clinical setting (17). We considered a difference between the test and reference methods of ± 0.5 L/min as good. This limit is based on our

experience as clinicians working with adult patients in the perioperative and intensive care settings where a difference of ± 0.5 L/min, in most clinical situations, would not alter the management of the patient. In adult patients this difference is relatively small compared to BL CO but has to be reconsidered if applied in a paediatric population. However, a consistent positive or negative bias represents a systematic measurement error and a solution should be sought to further improve trueness (86).

Percentage error is defined as the standard deviation divided by the mean of the corresponding values of the reference method. This entity gives additional information by relating the spread of data to the magnitude of the measured entity. If the standard deviation is constant, PE could be acceptable when evaluated in adults, but unacceptably high in a neonatal population. In paper I PE was > 45% for CO_{EPBF} and CO_{PAC} demonstrating that the spread of data in relation to the mean CO_{TS} was higher than both of the recommended limits of ± 30% and ± 45% (87, 88).

The article arguing for a PE of less than ± 30%, thus indicating that the reference method could be replaced by the test method, was written in 1999 by Critchley *et al*. (88). The ± 30% limit was derived from an assumed inherent precision of ± 20% for the clinical reference method i.e. thermodilution (PAC). The combination of an inherent precision of ± 20% for both the reference and test methods, results in a total error of ± 28.3%, which is commonly rounded up to ± 30% (83). However, when the inherent precision of the PAC has been measured in different experimental settings a larger variation has been display (97, 98). For example the number of bolus injections used to calculate CO alters the inherent precision of the PAC, 22% for a single reading and 13% when performed in triplicate (24). Furthermore, the haemodynamic alterations *per se* might influence PE. Significant haemodynamic alterations especially in high CO states might cause a larger spread of the measured data and a higher PE compared to during stable BL conditions (87, 90).

In a more recent publication by Peyton *et al.* evaluating CO monitors considered non-invasive, none of the included devices displayed a PE of < 30%, in fact the PE was around 45% for the majority of included methods (87). Based on these results the upper limit for an acceptable PE has been questioned and ± 45% has been suggested as a more realistic limit when thermodilution is used as reference method (87).

During comparable conditions PE decreased from 47% in paper I, to 34% in paper II at PEEP 5 cmH_2O before lavage. The size of the corresponding values for mean CO_{TS} could contribute to this finding. In paper I smaller animals with a lower CO were used compared to the larger sized animals with a higher mean CO in paper II. In all our papers we have included haemodynamic challenges and this has to be taken into account when interpreting the displayed PE.

THE EFFECT OF LUNG-LAVAGE ON AGREEMENT AND PRECISION

The agreement of CO_{EPBF} was negatively affected by surfactant depletion with high PE, wide LoA and impaired trueness. This finding is consistent with the fact that only the part of the pulmonary blood flow participating in gas exchange can be detected by a capnodynamic

method. The impaired agreement was especially prominent when the shunt fraction was > 20%. Due to the correlation between CO and shunt fraction, this finding was more pronounced during inotropic stimulation. The lower shunt fractions seen during preload reduction with subsequent lower CO displayed a better agreement.

When the direction of change of the paired delta values were analysed, trending ability was mostly preserved in spite of surfactant depletion. However, when the same paired delta values were analysed including the magnitude of change and the calibration of the test method, the concordance rate was decreased and the mean polar angle was high, indicating suboptimal calibration of CO_{EPBF}.

The underestimation of CO by CO_{EPBF} after surfactant depletion with subsequent increased shunt fractions is consistent with the fact that the method in the present form does not include a correction factor for shunt flow.

EFFECTIVE LUNG VOLUME

Since EPBF is included in the capnodynamic equation it could be presumed that significant changes in CO will affect the stability of ELV. Brewer *et al.* have also previously highlighted this although no data were presented (99). However, in in our study, ELV remained stable despite significant haemodynamic alterations within each PEEP level.

A small difference between methods was detected when PEEP was adjusted to 5 cmH_2O before surfactant depletion and at 12 cmH_2O after surfactant depletion. Surfactant depletion decreased the numerical values of both ELV and FRC_{PEEP} indicating a decreased lung function. Furthermore, the difference between ELV and the reference method increased with higher shunt fractions and was statistically significant at PEEP 5 cmH_2O after surfactant depletion. This presumably reflects that ELV estimates a functional lung volume dependent on the matching of ventilation and perfusion. In contrast, FRC_{PEEP} measures a static lung volume that reflects the volume in the lung into which the tracer gas disperses. However, the difference was small and the correlation was high suggesting that ELV could be used as a proxy for FRC during alterations of CO and lung mechanics (Figure19).

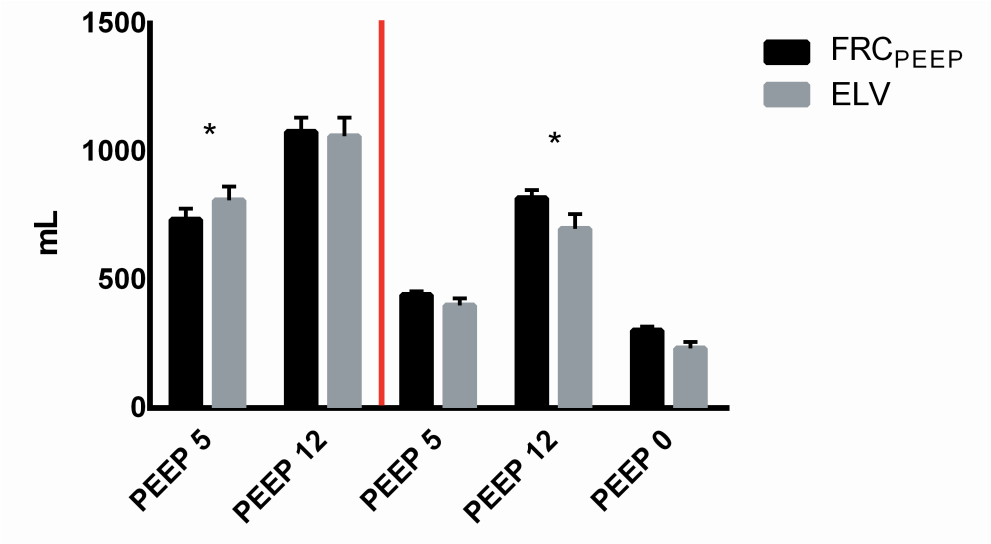

Figure 19. Functional residual capacity with a PEEP applied (FRC_{PEEP}) (mL) obtained by the SF6 technique and the effective lung volume (ELV) (mL) at different PEEP levels before and after lung injury was induced by multiple lavages (red line). There were significant differences between the volumes measured by the two methods within the PEEP steps in PEEP 5 cmH_2O before lavage (p=0.02) and PEEP 12 cmH_2O after lavage (p=0.03) using a paired t-test. Data are presented as mean (± SEM) (N=9).

PARADOXICAL INCREASE IN CO_{EPBF} AT HIGH PEEP

The unexpected paradoxical rise in CO_{EPBF} when PEEP was increased from 5 to 12 cmH_2O required further analysis. The mathematical formula is complex and includes several assumptions. When analysed in combination with the ventilation pattern based on inspiratory holds affecting the pulmonary blood flow, data simulation suggested that this overestimation of EPBF was enhanced by the inspiratory holds (unpublished data).

This finding is in line with previously described potential sources of error in rebreathing methods since alterations of the pulmonary blood flow *per se* may increase the differences between p_ACO_2 and end-capillary carbon dioxide (100). A ventilation pattern based on inspiratory holds may enhance the variation of intra pulmonary pressures especially at high PEEP levels. A pattern based on expiratory holds could be assumed to influence these pressures to a lesser extent. Additionally, a ventilation pattern with cyclically reoccurring inspiratory holds also affects the systemic circulation, especially in hypovolemic states, an unfavourable attribute in the perioperative setting.

In paper IV we evaluated CO_{EPBF} with a redesigned ventilation pattern based on expiratory holds during haemodynamic and lung mechanic alterations. No paradoxical increase in EPBF was detected and the agreement, precision and trending ability was preserved or improved.

THE LENGTH OF A MEASUREMENT CYCLE AND RECIRCULATION OF CARBON DIOXIDE

The storage capacity of carbon dioxide is large compared to production in the tissues and extraction from the lungs. Therefore the mixed venous content of carbon dioxide could be assumed to be unchanged or at least vary very slowly (101).

As previously shown in computer-based studies, the cycle time when the measurement is performed in relation to the size of EPBF is important for the performance of the method (102). In our studies the corresponding time described in the data simulation studies is equal to the length of one measurement cycle (9 or 10 breathing cycles). Results from a computer study based on a mathematical model shows that if EPBF is high the cycle time is better kept relatively short, and when EPBF is low a short cycle time may be inadequate. It could be concluded that the rebreathing cycle has to be short enough to avoid recirculation preserving the carbon dioxide content in the mixed venous blood at a constant level but long enough to assure adequate equilibration between end-capillary carbon dioxide and p_ACO_2 (62, 102). This is important since a prerequisite for the capnodynamic equation is unaltered concentration of carbon dioxide in mixed venous blood just before and during the rebreathing period for each measurement cycle. In our studies this time frame corresponds to the measurement cycle.

Even though no recirculation is present it seems like carbon dioxide rebreathing techniques even out EPBF and results in underestimation of EPBF when CO is high, as during inotropic stimulation, and overestimation of EPBF when CO is low (102).

These findings are consistent with our results from paper I and at PEEP 5 cmH_2O before surfactant depletion in paper II. Especially during inotropic stimulation in paper I where CO_{EPBF} underestimates CO and even shows a negative trend when the three subsequent measures are compared. In low CO states, as during caval occlusion and haemorrhage, CO_{EPBF} overestimates CO slightly. Our results from paper II showed that the shunt fraction varies with CO. This finding contributes to the underestimation of CO during haemodynamic interventions, as the capnodynamic method does not include shunt flow.

ESTIMATION OF THE END-CAPILLARY CONCENTRATION OF CARBON DIOXIDE

A differentiated Fick's equation includes an approximation of the end-capillary concentration of carbon dioxide from the actually measured concentration in the alveoli. A coefficient, described in the introduction, for carbon dioxide is added into the equation to convert the partial pressure of carbon dioxide to a concentration. In our studies an approach to this suggested by Capek and Roy was used (63). All approximations could involve potential sources of error. Alternatives approaches for conversion of partial pressure of carbon dioxide to concentration have been described, for example by Peyton *et al.* in 2001 (103). Optimisation of the approach to convert the solubility coefficient could provide an option to improve trueness.

CARDIAC OUTPUT MONITORING

We have high expectations of the ideal CO monitor; it should be non-invasive and safe, reliable with good agreement and trending abilities. Furthermore, it should be robust, continuous and easy to use with a short response time as well as hygienic and non-expensive. Unfortunately, no CO monitor has all the described qualities, in contrast it seems like they all have drawbacks (3, 25, 37, 38, 48, 51-53, 69, 104, 105). The non-invasive, easy to use CO monitors are unreliable when the circulation is unstable and the most accurate are invasive, potentially harmful and could be considered complicated to use (25, 53).

When assessing a CO monitor multiple factors have to be considered. Not just the precision of the method *per se* but also the precision and performance of the reference method. Percentage error and LoA could be expected to improve in an experimental setting with a stable haemodynamic situation (87). This described complexity may contribute to the diverging results from the numerous studies comparing two or more CO monitors.

In our studies the haemodynamic alterations were severe in order to evaluate the method in a way that is not possible in human studies. In the perioperative setting, haemodynamic shifts can be pronounced and rapid as in profuse haemorrhage with subsequent transfusion therapy. Consequently, the trending ability and response time are important properties of a feasible perioperative CO monitor. In paper I we have shown that CO_{EPBF} has a prerequisite for a fast response time. Data were collected from the sampling system from one animal during preload reduction and the response time in this example was 30 seconds.

A common situation in the perioperative setting is isolated haemodynamic instability without prominent lung pathology. Considering CO_{EPBF} shows similar performance to the clinical reference method for CO this method could be considered an alternative for CO monitoring in patients with isolated haemodynamic instability without significant lung pathology. The trending ability was also high when evaluated with the breathing pattern based on expiratory holds.

The choice of CO monitor should always be individualised and adjusted to the patient. The risk/benefit profile of available monitors should be weighed against the condition of the patient and the risk of complications. When the haemodynamic situation is severely impaired the requirement for agreement, precision and trending ability increases and a more invasive approach should be considered. The most invasive monitors with the opportunity for additive information, such as pulmonary vascular pressures and mixed venous saturation should be used for the most complex cases where a higher risk of the monitor *per se* could be justified by the benefit of monitoring and subsequent CO optimisation.

In summary, CO_{EPBF} showed reliable trending abilities and good agreement when evaluated with a modified ventilator pattern based on expiratory holds. That CO_{EPBF} was affected by lung-lavage when shunt fraction increased was an expected finding since there is no correction for shunt fraction. Furthermore, CO_{EPBF} could be considered non-invasive in mechanically ventilated patients, and continuous with a prerequisite for a short response time.

The capnodynamic equation estimates ELV, a lung volume factor that correlates to FRC (70, 106). A monitor combining assessment of EPBF and ELV could provide an interesting

possibility for optimisation of both CO and ventilator settings. Last but not least, the system seems to be safe to use in most patients (66).

CLINICAL IMPLICATIONS AND FUTURE PERSPECTIVES

Further evaluation of the modified ventilation pattern based on expiratory holds should be done after lung-lavage. The shunt fraction is not detected by a capnodynamic method regardless of ventilation pattern. Integration of a correction factor for shunt flow could improve agreement between CO_{EPBF} and CO.

The novel expiratory-based ventilation pattern should be evaluated in patients anaesthetised for high-risk surgery. Alterations of the concentration of carbon dioxide could be assumed to affect the performance of the method. Therefore, CO_{EPBF} should be evaluated during surgery when perturbations of the carbon dioxide concentration are common. This might occur after an ischemic period during vascular surgery or in the trauma patient after the tourniquet has been released. Another interesting field for clinical evaluation is during laparoscopic surgery. It is mandatory to inflate carbon dioxide into the abdomen to facilitate the surgeon's view. Since carbon dioxide equilibrates quickly with the tissue and blood this procedure might affect the performance of CO_{EPBF}.

The performance of CO_{EPBF} was evaluated when dead space was increased via a higher PEEP level. The paradoxical increase in CO_{EPBF} was not repeated when the modified expiratory breathing pattern was evaluated. However, alterations in the I:E ratio is likely to affect the pulmonary blood flow *per se.* Considering emphysema and hyperactive airways are common clinical pathologies animal studies are warranted.

Since the capnodynamic equation incorporates an assumption that the venous content of carbon dioxide is constant during a measurement cycle this should be validated. Collecting multiple mixed venous blood gases during a measurement cycle and comparing the carbon dioxide concentrations would be easy and could strengthen the evaluation of the method

There is a lack of a feasible monitor measuring both lung ventilation and perfusion. In paper III ELV correlated to the reference method for FRC and showed good trending abilities. Furthermore, ELV was found to be stable during CO alterations. Based on these results the entity ELV should be evaluated in anaesthetised mechanically ventilated patients.

A monitor of EPBF and ELV could provide an interesting option for optimisation of both entities. This has to be studied in relation to different breathing pattern.

CONCLUSIONS

In summary, we have evaluated CO_{EPBF} and ELV in a porcine model during haemodynamic and ventilator alterations before and after surfactant depletion.

The CO_{EPBF} method, based on inspiratory holds, showed good agreement and trending capability with high concordance rates and an acceptable mean angular bias when compared to the ultrasonic reference method and the CO_{PAC}. The overall agreement decreased slightly when CO_{PAC} was used as reference method whereas the trending ability was preserved.

The CO_{EPBF} method, ventilation pattern based on inspiratory holds, displayed a paradoxical rise compared to the ultrasonic method when PEEP was increased.

Lung-lavage induced significant lung injury and impaired agreement and precision of the CO_{EPBF} method. A significant correlation was found between impaired trueness and increased shunt fractions. Trending ability was largely preserved when concordance rate was assessed by the four-quadrant methodology. However, when assessed by the polar plot methodology the concordance rate decreased and the mean polar angle was wide indicating suboptimal calibration.

ELV was stable during CO manipulation and showed good accuracy, precision and trending capability when compared to the reference method at baseline conditions. Lung-lavage impaired agreement and precision whereas trending ability was preserved.

The CO_{EPBF} method, based on *expiratory* holds, showed an agreement and trending capability comparable with the ventilation pattern based on inspiratory holds. The precision as assessed by percentage error was improved and no paradoxical rise was detected when PEEP was increased.

“If you know algebra you can conquer the world”

Johan Sander

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